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SPINDLE DISAPPEARANCE AND CHROMOSOME BEHAVIOR AFTER PARTIAL-EMBRYO IRRADIATION IN CECIDOMYIIDAE (DIPTERA)

By

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With 18 Figures in the Text

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In early cleavage in *Cecidomyiidae* an elimination of chromosomes from somatic nuclei occurs, usually in the third, fourth or fifth division, depending on the species involved. The number of eliminated chromosomes (E chromosomes) is fairly constant. Metaphase of an elimination division is completely normal; all chromosomes split, and daughter chromatids separate. In anaphase the movement of all E chromosomes soon ceases, and they remain in the equator of the spindle. The other chromosomes (S chromosomes) reach the poles and are incorporated into daughter nuclei. After elimination is accomplished, all the somatic nuclei of the embryo have an incomplete chromosome set (2 S). Elimination fails to occur only in the nucleus of the mother germ cell, which at this time is already located in the pole plasm. In this nucleus all the chromosomes behave normally during the cleavage divisions, and it retains the full chromosome number (2 S + E), as do all of its descendants.

Experiments with the embryos of *Wachtliella persicariae* L. (GEYER-DUSZYŃSKA 1958, 1959), featuring the application of hair ligatures to the embryos, differential centrifugation of the embryos, destruction of various parts of the embryonic body by cauterization or destruction of single cleavage nuclei by irradiation with ultraviolet microbeams, have shown that the pattern of elimination is extremely constant. Whatever part of the embryo is destroyed, elimination always occurs in all somatic nuclei of the undamaged parts. If the nucleus of the mother germ cell is artificially retained in the somatic part of the embryo or shifted to this part, elimination occurs in this nucleus too. The only factor that inhibits elimination of E chromosomes and causes transformation of the elimination mitosis into a normal division is a homogeneous substance that stains deeply with hematoxylin. In untreated embryos this substance is localized in the meshes of the cytoplasmic

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reticulum of the pole plasm, that is, within this region from which the mother germ cell will arise. If this substance is artificially removed from the pole plasm, the elimination of E chromosomes occurs in the nucleus of the mother germ cell. On the other hand, if shifted into the somatic part of the embryo, it is able to suppress elimination in any somatic nucleus present by chance in its vicinity and thus to transform the elimination division of this nucleus into a regular mitosis.

Somewhat similar suppression of elimination in somatic nuclei was obtained by NICKLAS (1959) in paedogenetic embryos of *Miastor sp.* after centrifugation. The exceptional somatic nuclei retaining the full chromosome number were always located most posteriorly. NICKLAS deduced that posterior cytoplasm has a property of active inhibition of chromosome elimination. It seems to me that this property is not ascribable to the posterior cytoplasm as a whole but to one of its constituents, namely, the polar granules. NICKLAS has shown that the polar granules are composed of proteins and RNA. These granules are beyond doubt homologous to the homogeneous substance that stains deeply with hematoxylin in *W. persicariae* and to corresponding constituents present in pole plasm of other species of *Cecidomyiidae*. The experiments with centrifugation failed to provide information about their function, because in *Miastor* embryos the polar granules were not expelled from the posterior end as in embryos of *W. persicariae*.

NICKLAS states that the chromosome elimination is a largely autonomous act of the chromosomes and can be prevented but not initiated by cytoplasmic factors. My experiments with *W. persicariae* showed that the process of elimination is completely independent of the presence or absence of any part of the embryonic body, except the homogeneous substance that stains deeply with hematoxylin, and that the cause of elimination must be sought in the sudden change, probably chemical, that occurs in the direct vicinity of the E chromosomes during elimination division. According to the hypothesis previously advanced (GEYER-DUSZYŃSKA 1959), this change is induced by a set of elimination genes, probably located in the E chromosomes and producing some substance that injures their centromeres. This injury could consist either of some morphological change in the centromere itself or of a change in the mitotic spindle that suppresses its function. KRACZKIEWICZ (unpublished) also has stated that elimination occurs as a consequence of defects in centromere function. NICKLAS (1959) believes that the immediate cause of elimination must be sought in some failure in production of normal mid-anaphase tension, most probably because of the inactivation of chromosome fibers.

It seems that some decisive evidence on the actual mechanism of elimination could be obtained from the behavior of E chromosomes in

complete absence of spindle. As it is well known, the destruction of spindle in mitotic cells can be produced by various chemical treatments or by irradiation with ultraviolet light (c.f. ZIRKLE 1957, 1959). BLOOM, ZIRKLE and URETZ (1955) and ZIRKLE *et al.* (1956) obtained spindle destruction in *Triturus viridescens* after partial irradiation of cells in tissue culture with 8 μ heterochromatic microbeams. If a small part of the cytoplasm is irradiated the spindle fails to appear or, if already present, disappears. In such cells the chromosomes do not divide but their movement is not completely suppressed; they gather in a peculiar manner around a common central point, forming a so-called quasi-rosette. Afterwards the quasi-rosette separates into two daughter quasi-rosettes, each composed of undivided chromosomes segregated at random. The daughter quasi-rosettes move apart, and the cell divides. URETZ and ZIRKLE (1955) obtained inhibition of spindle formation or its destruction in irradiated blastomeres of the embryos of *Echinarachnius parma*. Here the irradiated blastomeres failed to divide.

The purpose of the present experiments was the destruction of spindle in *Cecidomyiidae* embryos.

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Material and Methods

Experiments were carried out on the embryos of *Rhabdophaga batatas* WALSH. The galls were collected in the vicinity of Chicago on low swamp willows. Adult females and males were bred from these galls in laboratory conditions. For experiments steadily ovipositing females were chosen. The development of embryos obtained from such females was perfectly normal; on the second day the segmentation of the embryonic shield was in progress, and on the third day the larvae hatched. The course of embryonic development was usually observed by dark field; a reflecting objective (50 $\times$) and a periplan Leitz ocular (8$\times$) were used. Photos were taken with a Leitz camera. Fixation was carried out on embryos stuck to the slide with Mayer's albumen. After puncture the embryos were immersed for 30—40 minutes in diluted Kahle fluid (glacial acetic acid + 95 per cent alcohol + formaldehyde + distilled water in the ratio 6:12:2:30), rinsed overnight in 90 per cent alcohol and stained with the Feulgen method (time of hydrolysis in n HCl, 10 minutes; temperature, 56° C). Slides were investigated with a phase-contrast microscope equipped with a Galileo 100$\times$ immersion objective and a periplan Leitz 8$\times$ ocular. Photos were taken with a Leitz camera.

For irradiations the ultraviolet microbeam apparatus developed by URETZ and coworkers (1954, 1957) was used, equipped with an AH-6 mercury lamp operating at normal pressure and a cobalt-nickel filter. Microbeams 8 μ and 16 μ in diameter were selected. The time of irradiation was 5—10 minutes for the somatic part of the embryo and 1—2 minutes for the germ cells. The penetration of ultraviolet to the chosen target inside the embryo was severely reduced by the presence of the two-layered chorion and the large amount of yolk. Mostly for these reasons work with microbeams finer than 8 μ in diameter was practically impossible;

successful irradiations were obtained only in exceedingly rare instances. Attempts at overcoming these difficulties were only partially successful. After complete dechorionation the embryo disintegrates immediately, but does not do so if only the outer layer is removed. If such a partially dechorionated embryo is immersed in 5 per cent dextrose solution the course of cleavage is perfectly normal, and such an embryo reaches the blastoderm stage in the same time as an untreated one. For embryos that were partially dechorionated, immersed in 5 per cent dextrose solution and flattened with a coverslip, the time of irradiation with 8 μ or 16 μ microbeams could be diminished to 2—5 minutes. The qualitative results of irradiation with these microbeams were completely identical for non-dechorionated and partially dechorionated embryos; therefore the data will be pooled in the following description.

Pattern of Cleavage

Cleavage begins about an hour after the egg has been laid. In the cytoplasmic regions surrounding cleavage nuclei large amounts of orange pigment are accumulated, so that their movements can be easily followed in living material. The stages of mitosis can be estimated from the shape of these regions: approximately in prometaphase they begin to elongate; in telophase they split transversely. In the first and second cleavages the nuclei move along the longitudinal axis of the embryo, in the third and following cleavages they spread laterally and move towards the surface of the embryo. Each mitosis is accomplished in approximately 15 minutes; the interphases do not exceed 20 minutes.

Investigation of fixed embryos revealed that elimination occurs in the fifth cleavage and follows the course typical of *Cecidomyiidae*. A regular metaphase plate is formed; all the chromosomes split longitudinally and begin the anaphase movement, but this movement soon ceases in all the E chromosomes. At the end of the anaphase, in the vicinity of each pole only 8 chromosomes are present; the remaining 20—30 chromosomes are left in the equatorial region (Figs. 5 and 6). After fifth cleavage all somatic nuclei of the embryo contain only 8 chromosomes (2 S), whereas in the germ-cell nuclei the full number of chromosomes (2 S + E) is present. The chromosome number for germ cells (2 S + E), observed in spermatogonial metaphase plates, was found to be 40.

In some individual embryos the elimination of chromosomes was observed to occur earlier than normal; in second division often 2 chromosomes are eliminated, in the third several, but in the fourth none.

The course of mitosis in the nucleus of the mother germ cell is always normal. In the pole plasm a large amount of granular polar substance is present (Fig. 1).

Special attention was paid to the appearance and shape of spindles. In all cleavages the spindles are well developed, the asters from middle prophase to early anaphase are distinctly visible, in metaphase the spindle body is very prominent, and anaphase movement is accompanied

by considerable elongation of the intercentriolar distance (Figs. 2, 3 and 4). The appearance and behavior of the spindle in elimination divisions follow the usual pattern, the only difference noticed being the larger equatorial diameter in anaphase and telophase. This difference is correlated with a greater lateral distribution of daughter E chromosomes within the equatorial region and by the presence of spindle fibers connecting them with the poles (Figs. 5 and 6).

Results of Irradiation

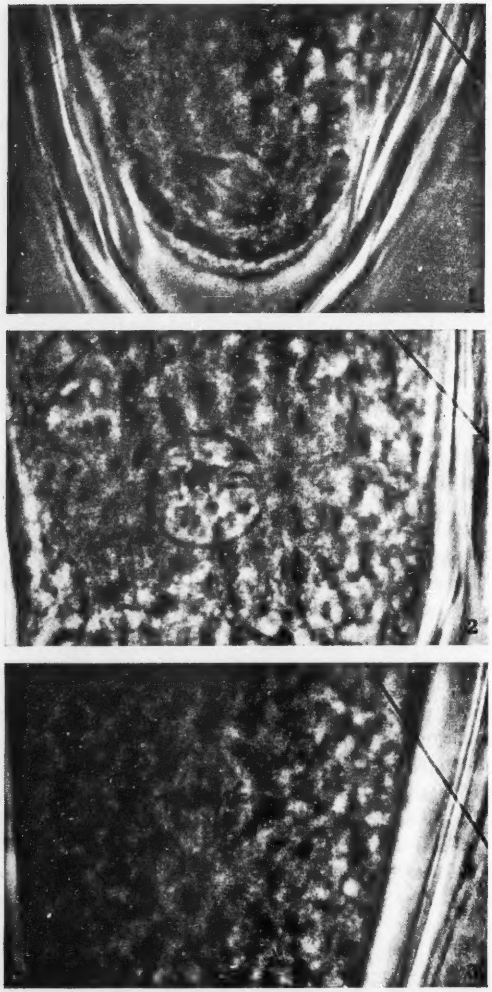
a) General remarks

For irradiation studies two divisions were chosen: the fourth cleavage, in which elimination was never observed and the fifth, in which elimination of all E chromosomes always occurred. In each experiment the embryo was carefully observed during the later stages of the preceding division, and irradiation was started in the following interphase at such a time that it was finished sometime during the prophase or metaphase of the division to be studied. The microbeam was focused on a part of the cytoplasm, and the cleavage nuclei were outside the target region (Figs. 7 and 8). In some embryos the irradiated cytoplasmic part was located posteriorly, in others medially or anteriorly. Irradiations of small parts of distal cytoplasm of mother germ cells were also carried out (Figs. 9 and 10). In some embryos, a small region of both somatic and germ-cell cytoplasm were irradiated. The experiments involved over 170 embryos. After irradiation the embryos were fixed either before the start of next cleavage or during the two following cleavages.

The effects of irradiation both in somatic and in germ-cell nuclei were multifold: complete degeneration of nuclei in the vicinity of irradiated cytoplasm; disappearance of spindle and formation of quasi-rosettes; multipolar mitosis of various configurations. The pattern of degeneration also varied greatly: in some embryos the nuclei were shrunken and strongly Feulgen-positive; in others they had the shape of enormous Feulgen-negative vesicles; in still others they were disintegrated into swarms of many small nuclei.

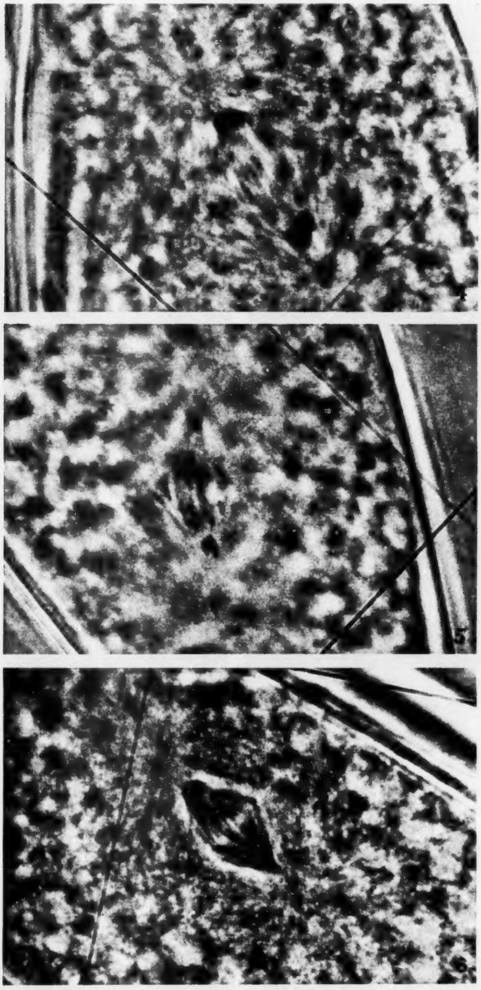
b) Spindle disappearance

Spindle disappearance was always followed by peculiar chromosome movements resulting in the formation of quasi-rosettes. These quasi-rosettes looked alike in the embryos irradiated in fourth division, fifth division and in germ cells (Figs. 11—18). No trace of spindle fibers was detectable; all the chromosomes were gathered in one big quasi-rosette or into two smaller ones. Closer analysis of the shapes of individual chromosomes in the divisions fixed while two daughter quasi-rosettes



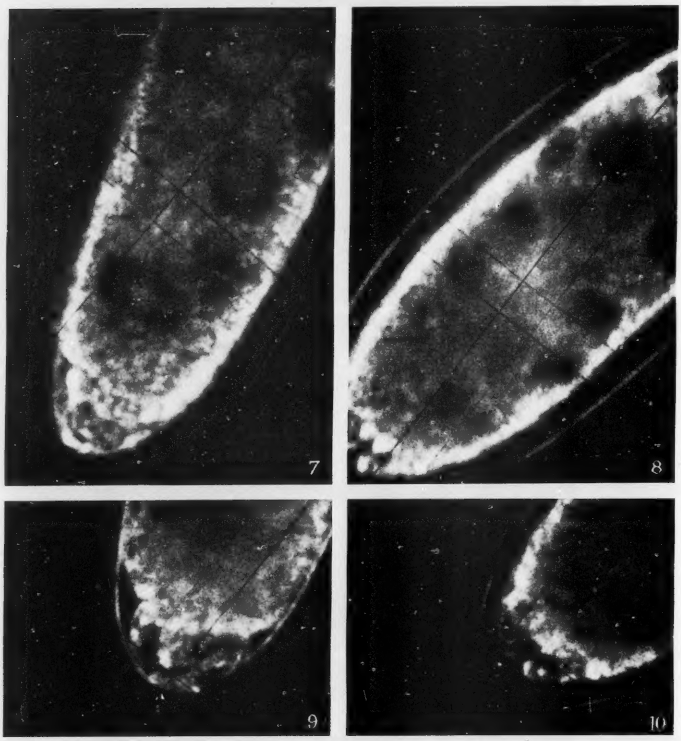
Figs. 1—3. Mitotic divisions in untreated embryos (immers. objective 100 ×, ocular 8 ×). Fig. 1. Anaphase in the nucleus of mother germ cell (the latter not yet constricted). Polar substance clearly visible. Fig. 2. Prophase in somatic nucleus. Fig. 3. Metaphase in somatic nucleus

were separating showed that the moving chromosomes were V- or J-shaped as in normal anaphase (Fig. 12). In irradiated divisions (both



Figs. 4—6. Mitotic divisions in untreated embryos (immers. objective 100 ×, ocular 8 ×). Fig. 4. Anaphase in somatic nucleus. Figs. 5 and 6. Chromosome elimination, anaphase

the fourth and the fifth) the E chromosomes behaved exactly in the same manner as S chromosomes, and there could be no doubt that with

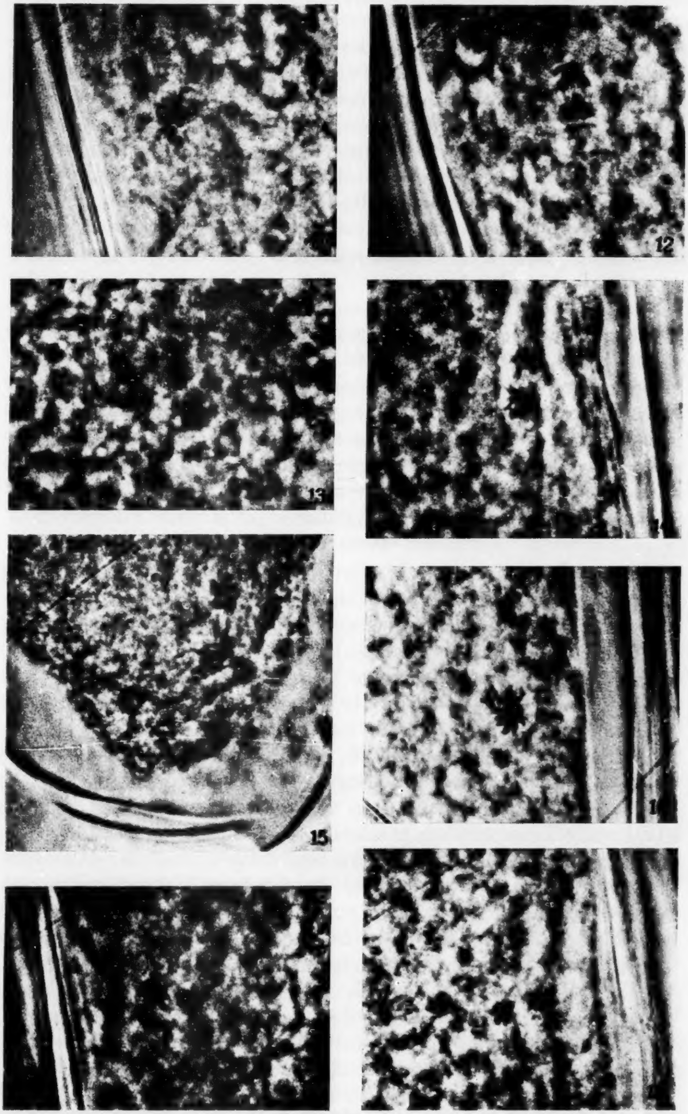


Figs. 7—10. The pattern of irradiation. Irradiated area circumscribed by cross-hairs (reflecting objective 50 ×, ocular 8 ×). Fig. 7. Embryo irradiated in fourth cleavage. Fig. 8. Embryo irradiated in fifth cleavage. Fig. 9. Mother germ cell, small part of cytoplasm irradiated. Fig. 10. Two germ cells. In the lower only a small part of cytoplasm irradiated; in the upper also part of the nucleus

the disappearance of spindle the difference in behavior between E and S chromosomes in the fifth division has vanished completely.

If in an embryo the spindles disappeared, they were always absent in all dividing nuclei in the vicinity of the irradiated region, and not infrequently all the somatic nuclei of such an embryo were deprived of

Figs. 11—18. Quasi-rosettes (immers. objective 100 ×, ocular 8 ×). Figs. 11—14. From embryos irradiated in fourth division. Fig. 11, single quasi-rosette; Figs. 12—14, daughter quasi-rosettes. Fig. 15. Daughter quasi-rosettes in the nucleus of mother germ cell. The polar substance severely damaged, its fragments on the right side. Figs. 16—18. From embryos irradiated in fifth division. Fig. 16, single quasi-rosette; Fig. 17 and 18, daughter quasi-rosettes



Figs. 11—18

spindles. In some embryos both quasi-rosettes and normally dividing nuclei with well developed spindles were present, e.g., in an embryo irradiated in fifth division and in the anterior part, one might see chromosomes gathered into quasi-rosettes nearby, while in the posterior half normal mitotic nuclei with regular spindles in telophase of elimination division were present. Quasi-rosettes in one embryo often were in different stages of division, some were still single, others already separated into two daughter quasi-rosettes. The formation of two telophase nuclei from daughter quasi-rosettes was observed; such nuclei never divide again, but become strongly Feulgen positive and degenerate in next divisions.

In germ cells in which spindle disappeared the cytokinesis was completely suppressed. If a mother germ cell was not split off at the time of irradiation, but only constricted, the constriction became less prominent after irradiation or even ceased completely. The polar substance was often severely damaged and fragmented (Figs. 1 and 15); such cells never divided. As the doses sufficient for production of mitotic aberrations were more than sufficient for inhibition of cell division, attempts to obtain germ cells with changed chromosome number were not successful.

Discussion

The experiments described above have shown that after irradiation in those mitoses in which spindles are absent the difference in behavior between E and S chromosomes completely disappears; in the time when elimination of E chromosomes usually occurs, namely in fifth cleavage, the activity of the centromeres of E chromosomes does not cease and the E chromosomes move in exactly the same manner as S chromosomes do until the very end of the division.

Thus, since E chromosomes can move actively in complete absence of spindle, it does not seem probable that their elimination is to be explained in terms of a lack or an inactivation of chromosomal spindle fibers. Such an explanation is also not in accordance with the fact that, in untreated embryos, in anaphase and telophase of the elimination divisions, spindle fibers connecting the daughter E chromosomes with both poles are clearly visible (Figs. 5 and 6). Moreover, in untreated cells of other *Cecidomyiidae* the behaviour of E chromosomes in various stages of meiosis is completely independent of the fate of the spindle. For example, in spermatogenesis of *R. batatas* chromosomes move actively in complete absence of spindle and segregate into two groups, S and S+E (GEYER-DUSZYŃSKA 1961). Also, in *Mikiola fagi* MATUSZEWSKI has shown (1959) that in oogenesis both the S chromosomes (bivalents) and the E chromosomes (univalents) are at first arranged on a common spindle, but then all the E chromosomes are eliminated

from this spindle, move actively for a while and then become arranged on a separate spindle; in metaphase of the first meiotic division the E chromosomes are included into the spindle in which the S bivalents are present. From these various facts it can be concluded that high autonomy of chromosome movement and the differences in behavior of S and E chromosomes are not peculiar to elimination cleavage but are general traits of the chromosomal cycle in *Cecidomyiidae* and are connected not with spindle structure but with intrinsic properties of the chromosomes.

Another explanation of elimination, based on intrinsic chromosomal properties, is that it occurs as a consequence of defects in the activity of centromeres of E chromosomes. There are two variants of this idea, one that these defects consist of morphological changes in the centromeres themselves, the other that changes in adjacent cytoplasm are injurious to the centromeres. The first possibility can be rejected on the basis of the present experiments: since the centromeres of the E chromosomes were active in changed conditions, it does not seem probable that any intrinsic change in these chromosomes occurs in the course of the fifth division. The second possibility remains and is strongly supported by the present experiments. As we have seen, when spindle disappearance is induced by ultraviolet irradiation, the capability of E chromosomes to move actively is restored. Spindle disappearance is not produced by direct action of ultraviolet on spindle fibers but results from irradiation of a more or less remote part of the cytoplasm. Zirkle and co-workers (cf. Zirkle 1959) explain this indirect effect in terms of a "spindle 'poison' formed by ultraviolet photochemistry from some cytoplasmic constituent". In *Triturus viridescens* cells spindle disappearance is accompanied by another indirect effect of ultraviolet irradiation, namely, suppression of chromosome separation into daughter chromatids. In *R. batatas* embryos also, this second indirect effect occurred in all mitoses in which the spindles were destroyed. Moreover, in such conditions the normally occurring disturbances in function of centromeres of E chromosomes did not occur; these chromosomes moved in exactly the same manner as S chromosomes and were incorporated into the daughter nuclei. The factor which prevented their elimination in the fifth division in irradiated embryos was an indirect action of ultraviolet light, i.e., a changed chemical surrounding. The supposition that the suppression of centromere function of E chromosomes is due to chemical change occurring in adjacent cytoplasm was originally based mainly on the fact that the only factor known to inhibit elimination was the presence of a homogeneous substance, deeply staining with hematoxylin, in the close vicinity of the dividing nucleus. Now a second such factor has been found — the indirect action of ultraviolet. The

mechanism of this indirect action is unknown; it is obvious that it somehow interferes with the occurrence of the change that normally causes elimination. In connection with my earlier hypothesis that elimination is due to the action of special elimination genes that produce some substance injurious to the centromeres of E chromosomes, a plausible assumption is that this production is in some manner disturbed by indirect action of ultraviolet.

Another interesting fact is that the polar substance also is highly susceptible to irradiation; in *R. batatas* embryos its partial destruction and fragmentation were observed in the same cells in which the spindle was destroyed. However, it is not clear whether these two effects of irradiation are due to the same indirect mechanism or result from two independent sequences of events.

Recently there have been reports of some cases of peculiar chromosome behavior in changed chemical conditions. BROWN and BENNETT (1957) found that in *Pseudaulacaspis pentagona* sex determination is connected with elimination of one haploid chromosome set in male embryos during early cleavage. Sexual dimorphism and dichronism manifest themselves as early as egg production; the female at first produces coral-colored eggs from which females are hatched, then pinkish-white eggs which give rise to males. Chromosome elimination occurs only in these latter eggs. Aging prior to mating results in marked increase in proportion of sons; a similar effect was obtained by irradiation of females with X rays. The authors conclude that the change in kind of eggs produced is due to physiological processes occurring in the ovaries; when these processes reach some threshold the production of the male eggs starts. Both aging and X rays act as accelerating factors. In this organism chromosome elimination results directly from the changes in the physiology of the egg cell as a whole, connected with prominent changes in its chemistry, as indicated by the change in kind of pigment produced.

SCHRADER (1960) has reported a somewhat similar case of differential chromosome behavior in changed chemical condition not however connected directly with chromosome elimination. In the harlequin lobe of some *Pentatomidae* spermatogenesis undergoes bizarre deviation from normal meiosis. The harlequin lobe differs greatly from other parts of the testes in its chemical composition, namely in the amounts of proteins and RNA present, the ratio of DNA to histones and the amino-acid composition of histones. In these conditions, pairing and formation of bivalents in isopycnotic autosomes is seriously disturbed; in metaphases they form large aggregations lagging in the equator of the spindle and are all incorporated into the same spermatid. At the same time the heteropycnotic chromosomes behave normally or show only slight

aberrations. SCHRADER concludes that this radically different behavior of heteropycnotic and isopycnotic chromosomes in a changed chemical environment can be evaluated as "a demonstration of the fact that chromosome behavior is to a considerable extent determined by their immediate environment".

Returning now to the elimination of chromosomes in *Cecidomyiidae*, it must be concluded that despite the fact that the experimental evidence as a whole indicates that elimination is caused by chemical change occurring in the adjacent cytoplasm in the course of elimination division, direct evidence of such a change is still missing. The cytochemical analysis carried out by NICKLAS (1959) on paedogenetic embryos of *Miastor* revealed that in the distribution of proteins, RNA and polysaccharides no significant changes up to mid-blastoderm stage occur. Moreover, gross gradients in the distribution of these substances do not exist. The same is true for the synthesis of DNA. These data would seem to indicate once more that chromosome elimination in the *Cecidomyiidae* ist not connected with changes in prominent features of the overall metabolism of the embryo and that more specific and localized chemical processes are here involved.

Summary

1. Currently available data on chromosome elimination in *Cecidomyiidae* are still inadequate to support any definite conclusions regarding its immediate causes. It seems that some evidence on the mechanism of elimination could be obtained from observation of the behavior of E chromosomes in complete absence of spindle. To bring about such absence of spindle, ultraviolet microbeams would appear promising, since they have already been used to destroy spindles in cells of other organisms.

2. For irradiation the embryos of *Rhabdophaga batatas* WALSH were chosen. It was established that in this species $2\,S + E = 40$ and $2\,S$ (for female) $= 8$. Elimination of 30-odd chromosomes regularly occurs in the fifth cleavage. Embryos were irradiated with $8\,\mu$ and $16\,\mu$ heterochromatic ultraviolet microbeams not only in this fifth cleavage but also in the fourth, in which elimination was never observed. The irradiated regions were small parts of somatic cytoplasm or small parts of germ cells.

3. The effects of irradiation were multifold: complete degeneration of nuclei in the vicinity of irradiated cytoplasm; disappearance of spindle and formation of quasi-rosettes; multipolar mitosis. The polar substance present in germ cells was often severely damaged and fragmented. All these effects are evidently indirect.

4. *In mitoses thus deprived of spindles, the separation of chromosomes* into daughter chromatids was suppressed but some of their active movements persisted. All chromosomes were gathered into a quasi-rosette, which afterwards divided into two quasi-rosettes. The daughter quasi-rosettes moved apart and formed telophase nuclei. In both irradiated divisions (fourth and fifth) E chromosomes behaved in exactly the same manner as S chromosomes; they showed the same kind of peculiar movement and were incorporated into telophase nuclei. Suppression of elimination of E chromosomes in the fifth division can be regarded as one more indirect effect of irradiation to be added to the list above.

5. These results show that elimination of E chromosomes does not result from failures in chromosomal spindle fibers or from intrinsic morphological defects in the centromeres. The fact that elimination can be suppressed by indirect action of ultraviolet light strongly supports the assumption that elimination occurs because of chemical changes in adjacent cytoplasm that are injurious to the centromeres of E chromosomes. Most probably the indirect action of ultraviolet interferes with the production of some substance that is normally injurious to the centromeres in the fifth division.

6. Since in *Cecidomyiidae* the general features of the metabolism are known to be stable up to the mid-blastoderm stage, it would appear that the chemical changes involved in elimination cleavage are probably quite specific.

References

BLOOM, W., R. E. ZIRKLE and R. B. URETZ: Irradiation of parts of individual cells. III. Effects of chromosomal and extra-chromosomal irradiation on chromosome movements. Ann. N.Y. Acad. Sci. **59**, 503—513 (1955).

BROWN, S. W., and F. D. BENNETT: On the sex determination in the diaspine scale *Pseudaulacaspis pentagona* (TARG.) *(Coccoidea)*. Genetics **42**, 510—523 (1957).

GEYER-DUSZYŃSKA, I.: Elimination of chromosomes in *Cecidomyiidae (Diptera)*. Bull. Acad. pol. Sci. Cl. 2 **6**, 371—375 (1958). — Experimental research on chromosome elimination in *Cecidomyiidae (Diptera)*. J. exp. Zool. **141**, 391—448 (1959). — Chromosome behavior in spermatogenesis of *Cecidomyiidae (Diptera)*. Chromosoma (Berl.) **11**, 499—513 (1961).

MATUSZEWSKI, B.: Chromosome behavior during oogenesis in gall midge, *Mikiola fagi* HART. *(Cecidomyiidae, Diptera)*. Bull. Acad. pol. Sci. Cl. 2 **7**, 101—104 (1960).

NICKLAS, R. B.: An experimental and descriptive study of chromosome elimination in *Miastor* spec. *(Cecidomyiidae, Diptera)*. Chromosoma (Berl.) **10**, 301—336 (1959).

SCHRADER, F.: Cytological and evolutionary implications of aberrant chromosome behavior in harlequin lobe of some *Pentatomidae (Heteroptera)*. Chromosoma (Berl.) **11**, 103—128 (1960).

URETZ, R. B., W. BLOOM and R. E. ZIRKLE: Irradiation of parts of individual cells. II. Effects of an ultraviolet microbeam focused on parts of chromosomes. Science **120**, 197—199 (1954).

URETZ, R. B., and R. P. PERRY: Improved ultraviolet microbeam apparatus. Rev. sci. Instr. **28**, 861—866 (1957).

—, and R. E. ZIRKLE: Disappearance of spindles in sand-dollar blastomeres after ultraviolet irradiation of cytoplasm. Biol. Bull. **109**, 370 (1955).

ZIRKLE, R. E.: Partial-cell irradiation. Advanc. biol. med. Phys. **5**, 103—146 (1957). — Structure of mitotic spindle. In Symposium on Molecular Biology, Univ. Chicago Press, p. 321—331, 1959.

— W. BLOOM and R. B. URETZ: Use of partial cell irradiation in studies of cell division. Proc. Int. Conf. Peaceful Uses Atomic Energy (Geneva) **11**, 273—282 (1956).

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PACHYTENE ANALYSIS IN ORYZA

II. STERILITY IN JAPONICA-INDICA RICE HYBRIDS

By

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With 32 Figures in the Text

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I. Introduction

KATO, KOSAKA and HARA (1928) classified the varieties of *Oryza sativa* L. into two subspecies, *japonica* and *indica*, based upon the hybrid sterility and morphological characters. Several of these inter-sub-specific hybrids have been subjected to cytological analysis with no evidence for structural hybridity, until 1952 when MELLO-SAMPAYO reported bridges at anaphase-I in some of these hybrids and his observation was confirmed by SAMPATH and MOHANTY (1954). HSIEH and OKA (1958) reinvestigated some of these hybrids and concluded that anaphase bridges occurred both in parents and hybrids and hence could not be considered as evidence for inversion heterozygosity. HENDERSON, YEH and EXNER (1959) likewise reported that chromatin bridges with fragments were very infrequent in the hybrids, but based upon the pachytene analysis of YAO, HENDERSON and JODON (1958), they concluded that these hybrids were heterozygous for inversions, but that these were either pericentric, or included, or the inverted segments non-homologously paired. Despite the limited cytological abnormalities, some of these sub-specific hybrids are as sterile as some of the inter-specific hybrids (range of sterility—10.42 to 99.9%, SHASTRY, unpubl.).

CUA (1952) and TERAO and MIZUSHIMA (1939) reported that *japonica* × *indica* allotetraploids are more fertile than the diploid hybrids, form quadrivalents less frequently and hence they suggested that cryptic structural differences existed between the genomes of the parents. OKA (1957), however, disagreed with this hypothesis, favouring random pairing at the tetraploid level since he secured tetrasomic inheritance for some loci studied by him. Thus, the nature of genetic differentiation between the sub-species and the causes of sterility in these hybrids were interpreted on purely genic models and it became necessary to postulate the operation of complementary polygenic systems (OKA 1953, 1956 and 1957) and pollen certation. Since these hybrids have not been adequately analyzed at diplotene and pachytene stages, the present study was confined mainly to these stages. A preliminary report of this study appeared elsewhere (SHASTRY and MISRA 1961).

II. Material and Methods

Four *japonica-indica* hybrids were secured by hybridization between the parents. Of the material employed in the study, Norin-6, A-18, 7942 and 7374 belong to the sub-species *japonica* while N.P. 130, N-32 and T. 21 belong to the sub-species *indica*. All the parents and the hybrids were diploids with $2n = 24$.

The spikelets of desired stages were fixed for 24 hours at 14^0 C in acetic-alcohol (1:3 by volume), to which traces of ferric chloride were added, transferred to 70% ethyl alcohol and stored till used. The anthers were squashed in a drop of 1% acetocarmine to which traces of ferric acetate were added. Temporary mounts were used for photomicrographs and line drawing of pachytene bivalents.

III. Observations

1. T. 21 (indica) $\times$ *A-18 (japonica),* F_1 *hybrid*

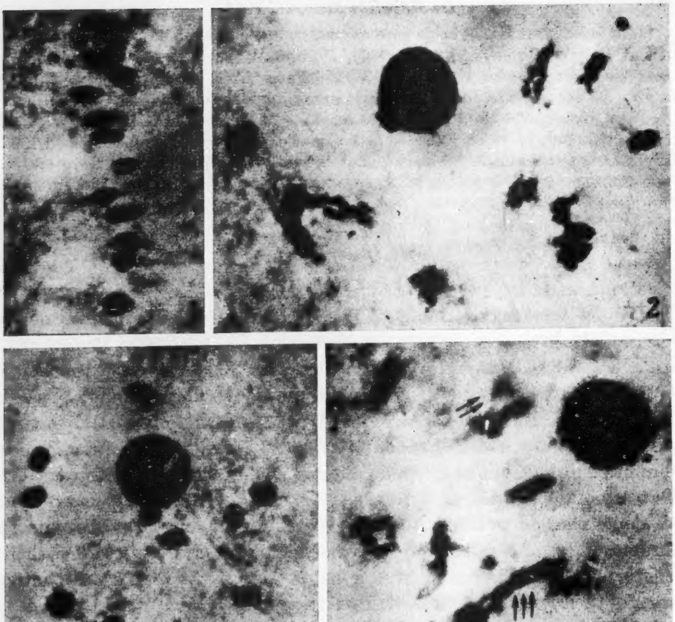
Pairing was normal in 64 of the 65 PMCs studied at diplotene in this hybrid with the occurrence of regular 12 bivalents. In one cell, however, a quadrivalent was recorded (Fig. 2). In every one of the 35 PMCs studied at diakinesis (Fig. 3), and in 38 PMCs at metaphase-I (Fig. 1), pairing was normal. Anaphase-I and anaphase-II, likewise, were completely normal, with no irregular numerical disjunction or chromatin bridges. Pachytene analysis of 25 analyzable PMCs revealed that although the bivalent formation was complete, every one of the bivalents showed pairing over only a part of the length of the pairing chromosomes. All the twelve bivalents could be identified by their length and arm-ratios and were numbered as 1—12 in the descending order of their length. A detailed description of each bivalent is as follows:

Chromosomes 2 and 12 were compactly paired, with no evidence of structural hybridity. The data on the chromosome lengths and arm-ratios of this hybrid are given in Table 1. Photomicrograph of one PMC with several abnormalities is given in Fig. 11. A diagrammatic representation of the karyotype, indicating the pairing in each bivalent, is given in Fig. 31.

Chromosome 1. Pairing in the short arm of this bivalent was normal. The long arm was paired normally all along, except at a distance of 9 μ from the centromere where a reversed repeat was noticed in one of the chromosomes of the bivalent. The duplicated region measured 4 μ with compact "fold back" pairing within itself giving a branch of 2 μ length (Fig. 14).

Chromosome 3. The short arm of this bivalent was normally paired, while the long arm exhibited two differential segments, one commencing close to the centromere and extending for 6 μ and the other in the terminal region for 5 μ length (Fig. 15). Further, the two chromosomes within the bivalent are of unequal length (2.5 μ difference).

Chromosome 4. The long arm of this chromosome was compactly paired all along its length, except for a small region commencing 4 μ



Figs. 1—6

from the centromere. The short arm was paired for a length of 6.5 μ from the centromere while the rest of the arm was differential and heteromorphic (Fig. 16).

Chromosome 5. Pairing was normal for most of the length. A terminal 'loosely paired' region of 4 μ length on the short arm, and similar region of 3.5 μ length on the long arm, were the only abnormalities recorded (Figs. 17 and 31).

Chromosome 6. The short arm was heteromorphic and was paired only for a length of 4 μ from the centromere. The long arm had a differential segment of 8 μ length commencing from the centromere. Chromomeric differences between the constituent chromosomes were apparent in both the differential segments (Fig. 13).

Chromosome 7. Pairing was normal for a length of 4.5 μ in the short arm and 4 μ of the long arm. The terminal regions of both the arms were differential but the telochromomeres of the short arm were synapsed. The unpaired region of the long arm appeared to be heteromorphic. The unpaired region of the long arm extended to 11 μ while that of the short arm extended to 3.5 μ (Fig. 18).

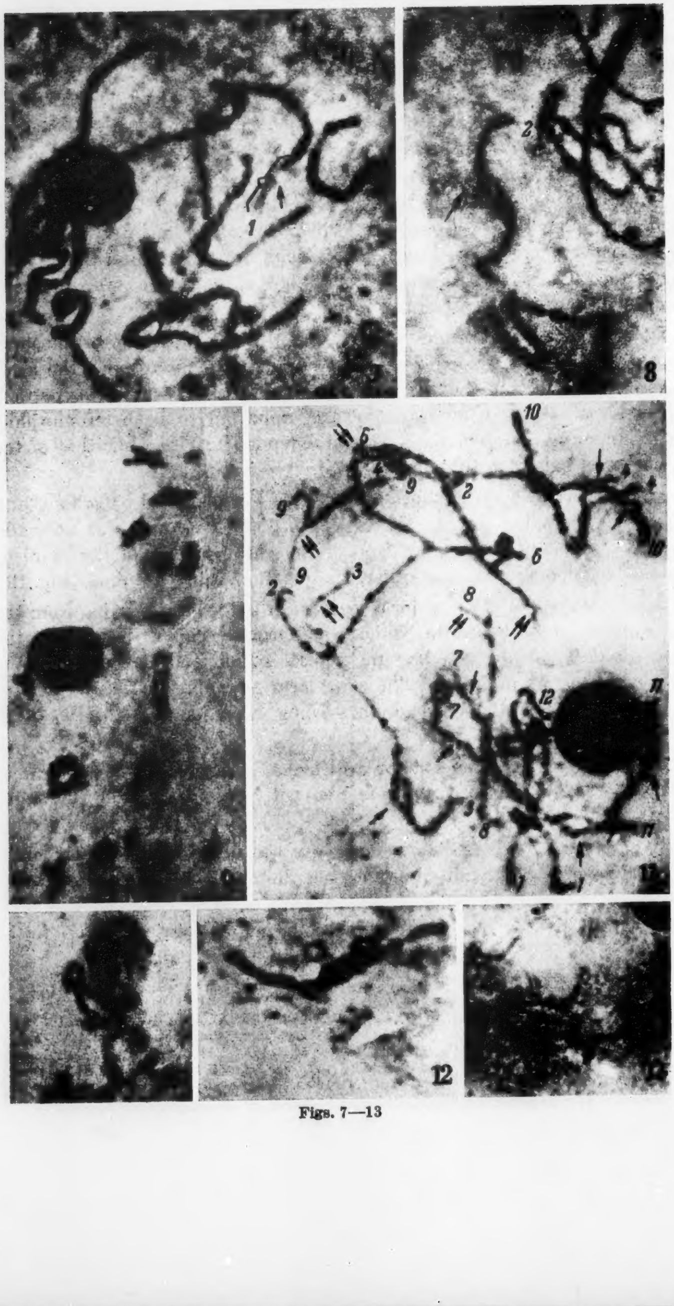
Chromosome 8. The short arm of this bivalent was 5.0 μ long and was loosely paired for 3 μ of its length. The long arm was normally paired all along its length. One of the chromosomes in the bivalent was 2.5 μ longer than the other, the deletion being terminal (Fig. 19).

Chromosome 9. Except for a segment of 9 μ length, starting from the distal end of its long arm, which was normally paired, the rest of the length of bivalent, including the centric region and the whole of the short arm, was unpaired. The short arm also appeared to be heteromorphic, one of the chromosomes being 1.5 μ longer than the other (Fig. 20).

Chromosome 10. Except for a differential segment in the middle of the short arm, this bivalent was compactly paired all along its length (Fig. 21).

Chromosome 11. The entire long arm was unpaired, one of the chromosomes of the differential segment of the bivalent passed through the nucleolus, while the other remained outside of it (Figs. 11 and 22). Further, this chromosome and chromosome 7 were observed to be involved in a small translocation (Fig. 12).

Figs. 1—6. Photomicrographs of pollen-mother-cells (PMCs). Figs. 1—3. Hybrid T. 21 × A-18. Fig. 1. Metaphase I, 8 ring-II and 4 rod-II. Fig. 2. Diplotene, 10 II + 1 IV. Fig. 3. Diakinesis, 12 II. Figs. 4, 5. Diplotene of hybrid N.P. 130 × 7942. Fig. 4. Large differential loop (↗), heteromorphic II (↗↗) and 1 IV (↗↗↗). Fig. 5. Heteromorphic II (↗) and 1 IV (↗↗). Fig. 6. Pachytene of hybrid N.P. 130 × 7374. Loose pairing (↗) in chromosomes *1*, *2*, *7*, *9*, and terminal differential segment in chromosome *5* (↗↗)

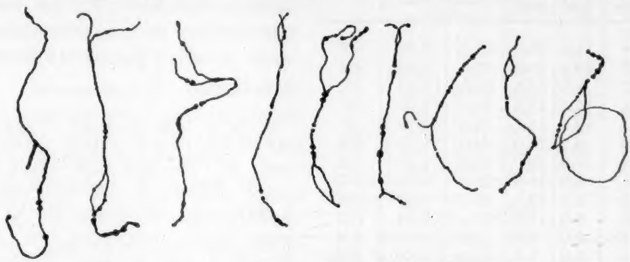


Figs. 7—13

2. N.P. 130 (indica) × 7942 (japonica), F_1 hybrid

In all the 20 PMCs studied at diakinesis and 25 at metaphase-I, regularly 12 bivalents were present. Regular 12—12 disjunction of the chromosomes with no evidence of either chromatin bridges or laggards was recorded in all the 30 cells studied at anaphase-I. Anaphase-II likewise, was normal thereby showing that all the gametes received a full complement of 12 chromosomes.

Diplotene and pachytene stages, in sharp contrast to later stages of meiosis, were irregular. Out of 29 PMCs studied at diplotene, regular



14 15 16 17 18 19 20 21 22

Figs. 14—22. Pachytene chromosomes in hybrid T. 21 × A-18. Fig. 14. Chromosome *1*. Fig. 15. Chromosome *3*. Fig. 16. Chromosome *4*. Fig. 17. Chromosome *5*. Fig. 18. Chromosome *7*. Fig. 19. Chromosome *8*. Fig. 20. Chromosome *9*. Fig. 21. Chromosome *10*. Fig. 22. Chromosome *11*. All chromosomes in Figs. 14—22 show abnormalities in pairing

12 bivalents occurred in 26 of them, while in the rest 3 PMCs, one quadrivalent in each due to what appeared to be small translocation was recorded. In one PMC (Fig. 5), one of the bivalents was heteromorphic. Further, the quadrivalent was composed of one heteromorphic and one homomorphic bivalents. In another PMC (Fig. 4), one quadrivalent composed of one large and one small bivalent was observed in addition to one heteromorphic bivalent. In the same cell another bivalent was observed where one of the chromosomes has a large differential loop. Another cell was found where a heteromorphic bivalent having one chromosome much longer than the other is attached to the nucleolus (Fig. 9).

At pachytene, only 5 PMCs could be analyzed and hence the karyotype has not been described in detail. The abnormalities recorded here

Figs. 7—13. Photomicrographs of PMCs. Figs. 7, 8. Hybrid N.P. 130 × 7374. Fig. 7. Pachytene showing loose-pairing regions in chromosome *1* (↗). Fig. 8. Differential segment in chromosome *2* (↗). Figs. 9, 10. Hybrid N.P. 130 × 7942. Fig. 9. Diplotene with unequal bivalent attached to nucleolus. Fig. 10. Terminal differential loop in a pachytene bivalent. Figs. 11—13. Hybrid T. 21 × A-18. Fig. 11. Pachytene, *12* II analyzable; *9* II show differential segments (↗) and heteromorphicity (↗↗). Fig. 12. Translocation heterozygote involving chromosomes *7* and *11*. Fig. 13. Differential loop in chromosome *6* (↗)

were similar to those in the hybrid, T. 21 × A-18. Large terminal differential segments were noted in some bivalents (Figs. 10 and 23). The entire short arm and the interstitial region of the long arm remained unpaired in one bivalent (Fig. 24). Regions close to the centromere were paired, while the distal regions of both the arms were differential in another bivalent (Fig. 25). In still another case, two bivalents were loosely paired (Figs. 26 and 27).

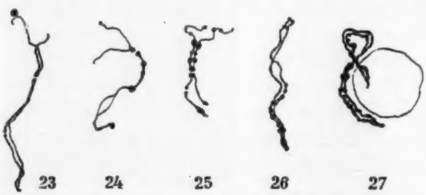
Table 1. *Lengths, arm ratios and classification of the chromosomes in F_1 of the two cultivated varieties of O. sativa, T. 21 × A-18 (indica × japonica)*

No. of the chromosome	Lenght of chromosomes in microns			Arm ratio; Short/long.	Classification *
	Short arm	Long arm	Total		
1	11.0	22.0	33.0	0.50	SM
2	10.5	20.0	30.5	0.49	SM
3	5.0	23.5	28.5	0.21	ST
4	12.5	12.5	25.0	1.00	M
5	10.5	13.5	24.0	0.78	M
6	9.0	15.0	24.0	0.60	SM
7	8.0	15.0	23.0	0.53	SM
8	5.0	15.0	20.0	0.33	ST
9	5.0	13.5	18.5	0.37	SM
10	6.0	11.0	17.0	0.54	SM
11	5.0	8.0	13.0	0.62	SM
12	5.0	7.5	12.5	0.66	SM
Total chromatin length			269.0		

* M = Median; SM = Sub-median; ST = Sub-telocentric.

3. N.P. 130 (indica) × 7374 (japonica), F_1 hybrid

At diplotene, diakinesis and metaphase-I, 50, 40 and 30 PMCs, respectively were analysed, all of which had regular 12 bivalents. In none of the 35 cells studied, either chromatin bridges or numerical abnormalities were noticed at anaphase-I. Detailed analysis of pachytene stage was undertaken in this hybrid. All the 12 bivalents were identifiable by their length, arm ratios and chromomeric pattern. Chromosomes 3, 4, 6, 8, 10, 11 and 12 were compactly paired all along their length with no evidence of structural hybridity. A special feature of the pachytene stage in this hybrid was 'loose pairing' in some regions in 4 bivalents (Figs. 6 and 7).



23 24 25 26 27

Figs. 23—27. Pachytene chromosomes in hybrid N.P. 130 × 7942. Fig. 23. Large terminal differential segment. Fig. 24. Short arm unpaired and interstitial differential loop. Fig. 25. Both arms unpaired except at centromere region. Figs. 26, 27. Two very loosely paired bivalents

Chromosome 1. This chromosome was 31.0 μ long and was median. Difficulties were encountered in the identification of this bivalent as the length variation from cell to cell was considerable due to loose pairing. In one PMC, the length of this bivalent was 25.0 μ, showing a considerable reduc-

tion in length due to loose pairing (Fig. 6). However, the arm ratio and the absence of definite chromomeric pattern enabled the proper identification of this bivalent. The proximal regions of both the arms for a length of 5 μ on either side of the centromere were compactly paired while the rest of the short arm and an interstitial 7 μ long segment were loosely paired (Fig. 7).

Chromosome 2. Distal ends of both the arms exhibited loose pairing upto a length of 3 to 4 μ. A differential segment was identified on the short arm commencing close to the centromere and extending upto 5.5 μ from it. The chromomeric pattern between the chromosome segments involved in this differential loop were not distinct (Fig. 8).

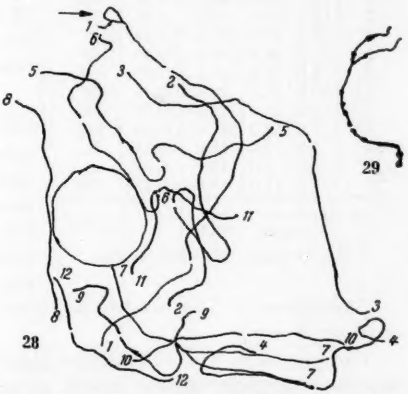
Chromosome 5. The short arm had a small (1.5 μ) terminal, differential region. Within this differential segment, one chromosome had three sharply-defined chromomeres, while the other had two chromomeres (Fig. 6).

Chromosome 7. This bivalent was loosely paired for the entire length of the short arm and a proximal 4 μ long segment of the long arm. The two individual chromosomes were observed to be loosely coiled throughout these regions.

Table 2. *Lengths, arm ratios and classification of the chromosomes in F_1 of the two cultivated varieties of O. sativa, N.P. 130 × 7374 (indica × japonica)*

Number of the chromosome	Length of chromosomes in microns			Arm ratio: Short/long	Classification*
	Short arm	Long arm	Total		
1	15.0	16.0	31.0	0.94	M
2	12.0	19.0	31.0	0.64	SM
3	5.5	23.5	29.0	0.23	ST
4	8.5	13.0	21.5	0.65	SM
5	7.0	12.0	19.0	0.58	SM
6	7.0	11.5	18.5	0.61	SM
7	7.0	9.5	16.5	0.73	M
8	8.0	8.0	16.0	1.00	M
9	3.5	12.5	16.0	0.28	ST
10	5.5	10.0	15.5	0.55	SM
11	5.5	8.5	14.0	0.64	SM
12	4.0	7.0	11.0	0.57	SM
Total chromatin length			237.0		

* M = Median; SM = Sub-median; ST = Sub-telocentric.



Figs. 28, 29. Pachytene of hybrid Norin. 6 × N-32. Fig. 28. Whole nucleus with terminal inversion loop (→) in chromosome *1* and unpaired long arm of chromosome *7*. Fig. 29. Chromosome *10* with terminal unpaired region

Chromosome 9. The short arm was almost completely loose-paired, while the terminal portion of the long arm was loosely paired for a length of 5 μ (Fig. 6).

A diagrammatic representation of the karyotype of this hybrid, indicating the nature of pairing of different regions is presented in Fig. 30. A tabular statement of the lengths, arm ratios and classification of the bivalents in this hybrid is given in Table 2.

4. Norin. 6 (japonica) × N-32 (indica), F_1 hybrid

Pairing was normal at diplotene, diakinesis and metaphase-I; at each of these stages, a minimum of 50 PMCs were analysed. Anaphase-I and Anaphase-II were likewise normal. Pachytene analysis was based upon the analysis of 20 PMCs with the complete complement analyzable and in some others where 5—6 bivalents were traceable. All the bivalents were identified and the data on pairing in each one presented below. Chromosomes 2, 3, 5, 8, 11 and 12 were completely normal while the others exhibited some abnormalities.

Table 3. *Lengths, arm rations and classification of the chromosomes in F_1 of the two cultivated varieties of O. sativa, Norin. 6 × N-32 (japonica × indica)*

Number of the chromosome	Length of chromosomes in microns			Arm ratio: Short/long	Classification*
	Short arm	Long arm	Total		
1	13.0	37.0	60.0	0.35	SM
2	16.0	29.0	45.0	0.55	SM
3	13.0	27.0	40.0	0.48	SM
4	17.5	19.5	37.0	0.89	M
5	17.5	18.5	36.0	0.95	M
6	9.5	16.5	26.0	0.57	SM
7	10.5	13.5	24.0	0.78	M
8	9,0	13.0	22.0	0.66	SM
9	7.5	13.5	21.0	0.56	SM
10	7.5	10.5	18.0	0.71	M
11	8.0	9.0	17.0	0.88	M
12	5.0	11.0	16.0	0.45	SM
Total chromatin length			362.0		

* M = Median; SM = Sub-median.

Chromosome 1. A sub-terminal inversion, involving a segment of 6 μ length was observed in the short arm of this chromosome at a distance of 2 μ from its end. In some preparations, this region remained unpaired (Fig. 28).

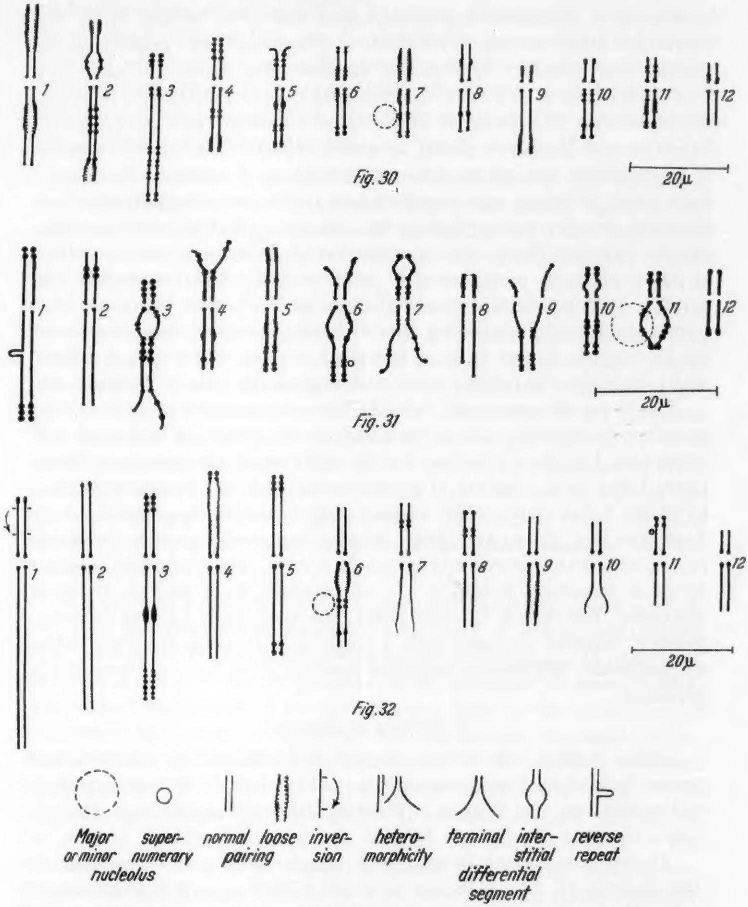
Chromosome 4. This bivalent was loosely paired all along the length of its short arm.

Chromosome 6. This chromosome organised the major nucleolus. The secondary constriction was clearly noticeable and was located at a distance of 7 μ from the distal end of the long arm. Between the secondary constriction and the centromere, this arm was loosely paired.

Chromosome 7. The entire length of long arm was unpaired. The short arm was normally paired and was attached to the minor nucleolus (Fig. 28).

Chromosome 9. A large (7.5 μ) loosely paired segment, was located on the long arm, 1.0 μ from the centromere.

Chromosome 10. The long arm had a large terminal differential segment of 5.5 μ length (Fig. 29).



Figs. 30—32. Diagrammatic representation of pachytene karyotypes of *Oryza sativa*-F_1-hybrids. Fig. 30. Hybrid N.P. 130 × 7374. Fig. 31. Hybrid T. 21 × A-18. Fig. 32. Hybrid Norin. 6 × N-32. Below, Fig. 32: Symbols used in the diagrams

A diagrammatic representation of the karyotype of this hybrid is given in Fig. 32. Data on the chromosome lengths and arm ratios of this hybrid are presented in Table 3.

IV. Discussion

1. Pairing at diakinesis and metaphase-I

Pairing at diakinesis and metaphase-I was quite normal in all the 4 hybrids—PMCs having 12 bivalents in every case. The present observations may therefore be regarded as confirming those of Hsieh and Oka (1958), Yao *et al.* (1958) and Henderson *et al.* (1959), who reported the occurrence of regular 12 bivalents at diakinesis and metaphase-I. Sampath and Mohanty (1954), however, reported that the occurrence of 12 bivalents can not be taken as indication of complete homology, since many bivalents were loosely paired and were even separated when excessive pressure was applied during squashing. In the present investigation, however, there was no reduction of chiasma frequency either at diakinesis or at metaphase-I in any one of the hybrids studied. In parents, 3—4 bivalents were rod shaped, while the rest were rings, the former presumably originating from the sub-telocentric chromosomes of the karyotype. In the hybrids, as well, 3—4 bivalents were rod shaped with a single chiasma, while the rest were ring shaped with 2—3 chiasmata.

The above discussion will indicate that no conclusive proof in favour of structural hybridity can be secured from the pairing at diakinesis and metaphase-I of these hybrids. On the other hand, the pairing at metaphase-I is a poor criterion of chromosomal sterility, cryptic structural hybridity being undetectable at that stage. Further, as pointed out by Stebbins and Vaarama (1954), cryptic structural hybridity which is more common than previously supposed, need not always be accompanied by gross structural hybridity. In an interspecific hybrid of *Melilotus* (Shastry, Smith and Cooper 1960), the analysis of pairing at metaphase-I, revealed no more than a single quadrivalent per PMC, while at pachytene, differential segments were recorded in every one of the bivalents.

2. Pairing at diplotene

Pollen mother cells of rice are well differentiated at diplotene and permit detailed analysis of associations and chiasmata. Heteromorphicity of the bivalents was clear in N.P. 130 × 7942, even at this stage thereby indicating that the parents differ in chromosome lengths.

During the present investigation, quadrivalents were identified in two hybrids (T. 21 × A-18 and N.P. 130 × 7942) in a low frequency of cells. While the occurrence of quadrivalents is an indisputable proof of the heterozygosity of these hybrids for translocation, the low frequency suggests that the regions involved are small, and are probably in sub-terminal positions and consequently are subject to terminalization.

None of the earlier investigators attempted the analysis of diplotene stages in these hybrids and, consequently, overlooked the occurrence of

quadrivalents due to small translocations. Even recently, YAO *et al.* (1958) based upon their own and earlier works concluded that the role of translocation, or restriction of homology between the inter-varietal chromosome complements, can be excluded in accounting for sterility. Unlike the previous reports, the present work clearly shows that translocated segments do occur, as revealed by the quadrivalents at diplotene. The discrepancy between the present work and the earlier ones is entirely due to choice of stage of analysis.

The observation of quadrivalents in intervarietal rice hybrids is particularly significant, since several times in the past it has been suggested that the karyotypes of the sub-species, *japonica* and *indica* were differentiated by accumulation of small chromosomal differences (TERAO and MIZUSHIMA 1939, KUANG 1951). More recently, however, this has been discarded in the construction of purely genic models to account for the sterility in these hybrids (OKA 1953, 1955b and 1957). OKA (1957), mainly based upon the regular bivalent formation and several other indirect cytological observations, concluded that a series of duplicate factors, are responsible for sterility. STEBBINS (1958) suggested that what are termed as G. D. genes by OKA might indeed represent small translocated segments. This view, however, was discarded by OKA, since no evidence for preferential pairing was obtained by him in the intervarietal amphidiploids. This present observation may well be a significant turning point in favour of chromosomal causes for sterility in these hybrids.

It is too well recognized that, however small the translocated segment might be and however infrequent the recovery of quadrivalent might be at metaphase-I, as long as pairing occurs at prophase and cross over takes place between the chromatids of the bivalents or quadrivalent, independent assortment of the chromosomes leads to the production of duplication-deficiencies in gametes. The occurrence of a single interchange of the type observed, indicates that for the segments involved in pairing, the genetic loci in the two parents occupy different positions on the linkage maps. If the segment is essential for the viability of the gametes, the sterility produced for this reason alone will be in the neighbourhood of 25%, as expected by the operation of G. D. genes. MIZUSHIMA and KONDO (1960) indicated that in some of the varieties belonging to the *indica* and *japonica* sub-species, the major anthocyanin locus, *Sp* might occupy different linkage groups, indicative of cryptic translocation differentiating these varieties. While they arrived at this conclusion by a purely genetic analysis, the present report of the occurrence of quadrivalents in these hybrids offers the cytological evidence.

3. *Chromatin bridges at anaphase-I*

Four inter-sub-specific hybrids varying in sterility from 43 to 76% were investigated for irregularities at anaphase-I. In none of these, chromatin bridges with or without fragments were recorded. This observation itself may not be taken as indication of negative role of inversions in the differentiation of these sub-species. For a typical chromatin bridge with a fragment to be recovered at anaphase-I, several conditions are to be satisfied: (i) The inversion must be paracentric; (ii) homologous pairing must occur within the segment heterozygous for inversion; (iii) a chiasma should occur within the inverted region. For the last condition to be satisfied, the segment involved in the structural change should be sufficiently large. Even in an interspecific hybrid, where heterozygosis for paracentric inversion involving $^1/_3$ of the arm was established by pairing at pachytene, chromatin bridges are recorded only in 25% of the PMCs analyzed at anaphase-I (Shastry *et al.* 1960). The present data, in the light of the above limitations of the anaphase studies, might therefore not be considered conclusive regarding the role of inversions in the differentiation of the sub-species.

Sampath and Mohanty (1954) reported the occurrence of chromatin bridges with fragments in 11 out of the 85 hybrids studied by them. Even in these hybrids, the bridges occurred only in a very minor percentage (data not given) of the PMCs. Mello-Sampayo (1952), likewise, inferred the role of inversions in the differentiation of the subspecies. Hsieh and Oka (1958), on the other hand, reported that these configurations occurred not only in the intervarietal hybrids but also in parents at comparable frequency and consequently are not indicative of structural hybridity. Yao *et al.* (1958) established the occurrence of inversions by the analysis of pachytene stages in these hybrids, but could not decide whether these were para-or peri-centric, since in their preparations the position of the centromere could not be determined with certainty.

Henderson *et al.* (1959) reinvestigated the problem of chromatin bridges at anaphase based upon the study of a large number of PMCs in 12 hybrids. In the hybrids that they have chosen for analysis, however, only two could be considered as highly sterile (28.7% and 34.4% pollen fertility), while others were only moderately so. They reported that chromatin bridges, not accompanied by fragments, occurred both in parents and hybrids and were consequently not due to inversion heterozygosity. The bridges with fragments occurred only in very small percentage (0—0.8%) of the PMCs, which led them to conclude that most of the inversions are either pericentric or the typical inversion loops were not formed at pachytene.

The present investigation offers a possibility to distinguish between these two alternatives. The pachytene analyses undertaken here, revealed in one hybrid a single case of sub-terminal inversion which is paracentric and in some PMCs the typical loop was not found, but only a differential segment was expressed. Differential segments involving the centromere were observed only in a single hybrid and in this too in a single bivalent. Even if it is considered that some of the differential segments owe their origin to heterozygosity for inversions, peri-centric inversions do not appear to be common in these hybrids.

HENDERSON *et al.* (1959), further suggested that belated separation of bivalents due to hindrance to chiasma terminalization (DARLINGTON 1937, pp. 510—11) might have been misinterpreted as chromatin bridges due to inversions. Similar "false bridges" due to sticking together of some of the chromatid ends were observed in grasshoppers (WHITE 1951). The recovery of fragments and the confirmation of pachytene pairing are the only two known means of avoiding this error. The granular fragments associated with bridges reported by HSIEH and OKA (1958) were considered by them to be non-chromatin inclusions.

In view of the above controversies with regard to the anaphase data in these hybrids and based upon the observations of the present study, it is possible that inversions have played a relatively minor role in the differentiation of these sub-species, although this conclusion might remain tentative until more hybrids are analysed. As would be obvious from the pachytene data to be discussed later, the cryptic structural hybridity need not be entirely visualized in terms of inversions of different types, as was done by YAO *et al.* (1958) and HENDERSON *et al.* (1959). Further, lack of positive proof of inversion heterozygosity may not be enough reason to exclude the role of cryptic structural hybridity and to support the genic causes for sterility as was done by OKA (1957) and HSIEH and OKA (1958).

4. Pairing at pachytene

In sharp contrast to the regular pairing at diplotene and later stages of meiosis, the chromosome pairing at pachytene was incomplete with evidence for structural hybridity in all the four hybrids studied. All possible structural changes—inversions, translocations, deletions and differential segments were recorded, clearly showing the extent of structural hybridity that is possible within the limits of regular bivalent formation. This will itself point out the inadequacy of metaphase-I and anaphase-I analysis for the elucidation of structural hybridity. These abnormalities clearly indicate that genetic differentiation at the intervarietal level progressed mainly by the structural changes and their

accumulation in different varieties was mainly due to the self-fertilization which serves as an effective mechanism of preserving these changes in hymozygous condition.

Duplications and Deletions. Duplications were mostly of the "reversed" type (Fig. 16). One of the chromosomes of the bivalent had a "branch" paired within itself and the thickness of this branch was comparable to that of the bivalent. The tandem repeat will exhibit a double loop and even if the double loop was not analyzable, the thickness of this region is expected to be double that of the bivalent. Such configurations were not observed in any of the hybrids. Reversed repeats were recorded in an inter-specific hybrid of *Melilotus* (SHASTRY *et al.* 1960) and in *Triticum* treated with vegetable oils (SWAMINATHAN and NATARAJAN 1959). Terminal deletions are the next common abnormalities recorded. In one of the parents small regions of 2—5 μ lengths were deleted. By the study of karyomorphology it was observed that some of the bivalents terminate in darkly stained regions within which chromeric details are not analyzable. While no relationship can categorically be suggested regarding the stainability and the genetic inertness, it is possible that at least a part of these might be considered as heterochromatic. If this was so, terminal deletions might owe their origin in such segments. Alternatively, the terminal regions of some of the chromosomes might have undergone translocation to other chromosomes of the complement.

Inversions. Inversions are the least common in all the hybrids studied. A single sub-terminal inversion loop was recorded in a bivalent of one hybrid, Norin 6$\times$N-32. In some PMCs a typical inversion loop was not formed, the constituent segments being non-homologously paired or exhibiting no pairing at all. TING (1958) likewise recorded that some of the sub-terminal inversion heterozygotes in maize exhibit all degrees of non-homologous pairing to total asynapsis. MCCLINTOCK (1933) recorded similar non-homologous pairing in some inversion heterozygotes. In such cases, neither the typical anaphase bridge is recovered nor the fertility is affected.

The role of inversions in the differentiation of *japonica* and *indica* varieties has been proposed by MELLO-SAMPAYO (1952), SAMPATH and MOHANTY (1954) and this has been disputed by HSIEH and OKA (1958). This controversy was mainly because the analysis was done at anaphase-I alone. YAO *et al.* (1958) confirmed the role of inversions by pachytene analysis, but the recovery of anaphase bridges at low frequency led them to conclude that most of these inversions are pericentric. The present investigation offers no support to such a view. Regions close to centromere are generally less exposed to structural changes. Even if it is assumed that some of the inverted segments do not exhibit loop-

formation, at least a differential segment might be expected in these cases, as has been determined for the sub-terminal paracentric inversions discussed above.

The present investigation clearly revealed that the differential segments do not include the centromere except in one bivalent in a single hybrid (T. 21 × A-18). It is therefore most likely that even pericentric inversions have played a limited role in the differentiation of these subspecies. SHASTRY (1958) reported occurrence of differential segments in seven out of eight bivalents in the hybrid *Melilotus messanensis* × *M. segitalis*, but none of these included centromere. The role of inversions in the differentiation of these two species as well, is of very minor importance. Even in plant genera like *Paeonia* (STEBBINS 1938), where the role of inversions was considered significant, pachytene analysis might reveal several other abnormalities not analyzable otherwise.

Loose pairing. Four bivalents in the hybrid, N.P. 130 × 7374, and some regions of 1 to 4 bivalents in other hybrids exhibited what are designated by the present authors as loosely-paired segments, the exact nature of which cannot yet by determined with certainty. If it is assumed that the homology is the property of the segments (Pairing blocks, DARLINGTON 1937) and not of the entire chromosomes, loose pairing might represent a highly complex structural hybridity. If the constituent chromosomes of the bivalents have undergone a series of duplications by unequal crossing over, the pairing blocks will be of limited size, interrupted with duplicated regions. There is abundant evidence to show that such duplications do occur (Bar locus in *Drosophila*; STURTEVANT 1925, STURTEVANT and MORGAN 1923). The occurrence of several included inversions (STURTEVANT 1938), as well, might lead to loose pairing, but this hypothesis was not favoured by the limited evidence for gross inversions in this material. Further, the variation in length between the chromosome complements of the species which is not accompanied by gross structural hybridity, *viz*. interstitial or terminal duplications, might be explained on the basis of several small duplications. At the observational level, the loosely paired segments are clearly analyzable by their hazy outline and lack of compact chromomere to chromomere pairing.

Translocations. In the hybrid, T. 21 × A-18, a quadrivalent was recorded at pachytene. Not only has this served as a confirmatory and decisive evidence regarding the role of interchanges in the differentiation of the chromosome complements of the parents, but has permitted the identification of the chromosomes (Nos. 7 and 11) involved in such an interchange. Further, one of the arms of the star-shaped configuration at this stage was completely unpaired, thereby indicating that subsequent to the occurrence of this interchange, the two homologous arms have

undergone further structural alteration leading to limited homology. In PMCs, where only bivalents were recorded, the occurrence of differential segments was in accordance to that expected on the basis of reciprocal translocation. Further, in such configurations, early terminalization or even non-occurrence of the quadrivalent is favoured since the homologous segments involved between the bivalents are small.

Differential segments. By far the most predominant form of structural hybridity noted in these inter-subspecific hybrids at pachytene was of differential segments. Since these configurations themselves do not lend any evidence regarding their nature, these could represent a highly heterogenous group, the significance of which, however, can not be belittled. Differential segments could serve as the building blocks of restrictions on homology between the chromosome complements and might therefore serve as effective mechanisms in genomic differentiation.

Two possible mechanisms can be visualized for the occurrence of differential segments. A major inversion followed by repeated included inversions or a single small inversion might lead to great reduction in the pairing blocks, thereby producing differential segments. The other possibility is the occurrence of small translocations. Fortunately, it is possible to distinguish between these two alternatives. If the region involved in a differential segment originated by inversions, where for reasons of size alone the typical loop is not expressed, it is expected that the genetic constitution of the two chromosomal regions involved in this segment is identical. Consequently, the viability of the gametes cannot be affected and this should have no influence on fertility. It is clear that the duplication-deficiency gametes are formed in the inversion heterozygote as a consequence of occurrence of crossing over within the inversion loop. On the other hand, if it is postulated that inversion leading to position effects are responsible for sterility, one of the parents should have been sterile. By way of both these lines of approach, it is most likely that most of the differential segments are not due to inversions.

The chromomeric pattern of the differential segments in some cases (chromosome 3 in the hybrid T. 21$\times$A-18) is, however, indicative of inversion heterozygosity, by the reverse arrangement of the chromomeres within the differential segment. Similar differential loops were recorded on *Oryza stapfii* (RAO 1960). These cases are, however, limited in number. In some differential segments, no differences in chromomeric pattern were noted. Although, chromomeric pattern is a poor criterion of true structural difference, these cases might cast some doubt upon the validity of their being true differential segments. Similar observations were made by RAO (1960) in a pure species, *O. glaberrima*.

In a large proportion of the differential segments, the chromomeric pattern is distinctly different in the constituent chromosome segments

of the bivalent. Considering the occurrence of small translocations at diplotene, the high sterility of these hybrids and the relatively minor role of inversions as discussed above, it is postulated that a majority of these represent cases of simple translocations. Unlike in the case of inversion heterozygotes, where the typical loop formation is a prerequisite for sterility, translocations irrespective of the size, and of the occurrence or otherwise of the quadrivalents, will lead to sterility by the independent assortment of the chromosomes.

In the hybrids with normal pairing at metaphase-I, and varying degrees of abnormalities at pachytene, it may be possible to predict the sterility of the hybrids by the degree of abnormalities at pachytene. It was felt that a suitable measure of abnormalities at pachytene was to be found to compare different hybrids. The length of the differential segments, loosely paired regions and deletions expressed as a percentage of the total chromatin length of the hybrid at pachytene, is designated by the present authors as "differential index". It will be obvious from the Table 4 that the hybrids differ considerably in their differential indices. While it is fully realized that the deficiencies in different locations might have different effects on viability, in general, this index might offer a possibility of predicting sterility of the hybrids. In hybrids

Table 4. *Differential indices and pollen sterility*

Hybrids	Total chromatin length involved in differential segments (in μ)	Total chromatin length of complement (in μ)	Differential index	Pollensterility (%)
T. 21 × A-18	83.5	269.0	31.04	76.44
N.P. 130 × 7473	43.5	237.0	18.31	46.01
Norin 6 × N-32	53.5	362.0	11.90	43.00

with varying type of gross structural hybridity, sterility might further be enhanced over that expected by hybridity measured by differential index.

The statement of STEBBINS (1950), that most of the so called cryptic structural hybridity remains cryptic only because the most suitable stage, *viz.* pachytene, is not possible for analysis in many plant genera, is amply substantiated by the present study. In the hybrid, *M. messanensis* × *M. segitalis*, likewise, the regularity of pairing is only at metaphase-I, while at pachytene, every one of the chromosomes exhibited differential segments (SHASTRY *et al.* 1960). This will also emphasise how small a region of pairing is adequate for persistent bivalent formation at metaphase-I.

5. Sterility and its genetic consequences

Sterility in hybrids is classified as—chromosomal, genic and cytoplasmic. While the last category is easily excluded by the studies of reciprocal crosses, the distinction between genic and chromosomal sterility becomes difficult in several plant materials and has to be based upon more indirect criteria—reduction in chiasma frequency in the primary hybrid and preferential pairing in amphidiploids. In genera, where pachytene analysis is possible, a part of chromosomal sterility not directly analyzable otherwise, is brought to an observational level. The major controversy of genic versus chromosomal sterility in the intervarietal rice hybrids is mainly a problem of technique and the analysis of proper stage of meiosis. Below the level of resolution of pachytene analysis, the distinction between genic and chromosomal sterility is theoretical as was evident from the inadequacy of pointing out the distinction between a true gene mutation and structural changes in irradiation experiments.

The present investigation offers a new orientation to the problem of sterility in *japonica-indica* rice hybrids. Small translocations that were observed at diplotene can lead to the production of 25% pollen sterility by the disjunction of the quadrivalents and by the independent assortment of the bivalents. Gametic development genes postulated by OKA (1953) in the light of the present study may well be interpreted as the homologous segments located on non-homologous chromosomes. A similar view was expressed by STEBBINS (1958), but it remained hypothetical in the absence of cytological data supporting it.

Differential segments, unravelled in the present study and which have been argued as possible sites of translocated segments, further offer hypothetical models which can explain high sterility in these hybrids. When the different regions of the bivalents exhibit alternate paired and unpaired segments and if it is assumed that the unpaired segments represent translocations, the occurrence of crossing over within the paired regions leads to an unbalance in genetic constitution leading to the duplication-deficiencies in gametic contributions. This mechanism will operate at the level of occurrence of bivalents. The density of the differential segments, in combination with the varying degrees of tolerance for deletions in different chromosomal regions, determines the extent of sterility. It will be obvious from the above that purely genic explanations for sterility have been proposed too prematurely and would have been supported only if the presumed normal pairing between the chromosome complements of the sub-species was realized at pachytene. In the light of a high degree of structural hybridity unravelled by the present study, purely genic explanation to account for sterility, are inadequate.

The role of inversion heterozygosity, likewise, which has been overemphasized (YAO *et al.* 1958), despite the limited cytogenetical evidence in favour of it, seems to be of limited value in accounting for the high sterility. As has been pointed out earlier, the low recovery of chromatin bridges accompanied by fragments distinctly eliminates their major role in accounting for sterility.

OKA (1957) considered that translocations might not have played significant role in the differentiation of *japonica* and *indica* types, mainly based upon indirect criteria. Despite the enhanced fertility of amphidiploids compared to primary intervarietal hybrids (CUA 1952, MASHIMA and UCHIYAMADA 1955), OKA (1957) concluded that preferential pairing does not occur at the tetraploid level, since he secured polysomic ratios for some loci (OKA 1955a). It will be obvious from the cytological data of these hybrids that univalents do not occur at meiosis, but the bivalents are unpaired for a part of their length. If such a hybrid was used for chromosome doubling, the nature of pairing at tetraploid level resembles that of a segmental allopolyploid. It is but normal to expect tetrasomic ratios for certain loci. A detailed analysis of several marker loci in the amphidiploids can alone give a genetic proof for preferential pairing. Alternatively, the pairing at pachytene of the amphidiploids can give direct evidence. In the absence of both these data, the evidence for preferential or random pairing in these hybrids is tentative. OKA (1957) used the lack of evidence in favour of preferential pairing as a support for the genic causes for sterility. He further emphasized the polysomic nature of the complementary factors implicated in sterility, and the present investigation clearly points out the multiplicity of the differential segments as an alternative approach to this problem.

The present investigation clearly suggests that the sub-species, *japonica* and *indica*, are differentiated by a series of small structural differences in their chromosome complements. Sterility in the hybrid progeny may therefore be looked upon as "recombinational". OKA (1957), likewise, postulated recombinational sterility, but his hypothesis was mainly based upon recombination between duplicate factors, while the present work suggests recombination between chromosome segments. Several observations on the inheritance of sterility in these crosses support the latter view. Very low parent/offspring correlation for sterility is the usual feature in all these hybrids. Highly sterile as well as highly fertile F_1 hybrids revealed the same mean and variation for sterility in F_2 generation. Inviable combinations are eliminated much earlier in the sterile hybrids than in the fertile ones, although in both cases they are (SHASTRY, unpubl.). To account for such divergent behaviour it becomes necessary to postulate the occurrence of several duplicate

factors, each operating at different levels and influencing fertility (G.D. genes, duplicate fertility factors, certation phenomena, etc., OKA 1953, 1955 and 1957). A more likely alternative indicated by the present investigation is the differential index of the hybrids and the probability of crossing over in some regions.

Non-recovery of recombinants. Non-recovery of full spectrum of recombinant phenotypes in the intervarietal rice hybrids is the next common observation which has been explained on the basis of linkage between the marker and the G.D. genes (OKA 1953). The present authors, on the other hand, are inclined to favour the view that the differential segments impose several restrictions on recombination. If it is postulated that at least some of the distinguishing phenotypic characters depend upon the chromosome segments involved in the differential loops, the cross over in the paired regions leading to elimination of most of the recombinant phenotypes, preserves the identity of parental phenotypes in the hybrid progenies. Further, the reduction in chiasma formation in the unpaired regions in the hybrids with higher differential index might lead to a corresponding increase in the chiasma formation in the paired regions. This may itself restore the parental combinations even in the absence of sterility.

Occurrence of abnormal plants. SHASTRY (unpubl.) recorded the occurrence of *albina* plants in low frequencies (0.1 to 0.6%) in F_2—F_5 generations of *japonica—indica* hybrids. VENKATSWAMY (1957) recorded the occurrence of plants with "narrow leaf", "dwarf sterile", "long sterile lemma", which were interpreted by him as a result of high "instability" leading to increased spontaneous mutation rate in the hybrid progenies. While it is realized that genotypes known to enhance the spontaneous mutation rate are not uncommon, the present cytological study permits a re-interpretation of these findings.

It would be obvious from the above reported characters that all of them are recessive. A deletion and point mutation are indistinguishable in an analysis of this type. It is, therefore, most likely that these abnormal plants might also owe their origin to minor deletions which are viable in the haplophase. If this hypothesis was true, it is easy to visualize the occurrence of gametes carrying deletions in cryptic structural hybrids with a high frequency of differential segments. Even with regard to the occurrence of abnormal plants, as in the explanations for sterility, a purely genic explanation is to be favoured only when the cytological data do not reveal any abnormalities indicative of structural hybridity.

6. Genetic differentiation between the sub-species, japonica and indica

This preliminary investigation permits some tentative conclusions regarding the nature of genetic differentiation between the sub-species *japonica* and *indica*. Considering the evolutionary age of the species, mode of reproduction (autogamy), varietal diversity and, wide range of eco-geographical conditions in which the species is grown, genetic differentiation between the varieties is expected to cover a broad spectrum. The main point is whether this is achieved by the accumulation of several gene mutations or by chromosome structural changes. Inadequate cytological analyses hitherto undertaken seemed to favour the former view.

Following a purely genetic approach, MIZUSHIMA and KONDO (1960) concluded that the varieties of the sub-species *japonica* and *indica* are differentiated by a small translocation involving *Sp.* locus. The present investigation revealed the occurrence of quadrivalents at early diplotene in 2 of the 4 hybrids studied. The major line of evidence of the relationship between differential index and sterility and occurrence of abnormal phenotypes in hybrid progenies indicate that small translocations have played significant role in the differentiation of the varieties of these sub-species. It is possible that the loci or the chromosomal segments occupying corresponding positions in the pre-*japonica* and pre-*indica* probably have been reshuffled into different positions in the evolution of the present day cultivated rices. Discrepancies in the genetic ratios occurring in several crosses when the parents involved are not from the same region are to be interpreted in this background. In future, genetic studies might have to be confined only to cases where pachytene pairing was established to be normal.

Summary

Pachytene analysis was untertaken in 4 *japonica-indica* rice hybrids. In all these hybrids, pairing was exceedingly abnormal lending evidence for structural hybridity. Earlier investigators who analysed metaphase-I and later stages of meiosis concluded that sterility is due to genic causes. The present investigation clearly points out the chromosomal causes of sterility and makes possible a reinterpretation of (1) non-recovery of recombinant phenotypes and (2) the occurrence of *albina* and other mutations in hybrid progenies.

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References

CUA, L. D.: Artificial polyploidy in the *Oryzae*. III. Cytogenetical studies on intra and inter subspecies tetraploid hybrids in *Oryza sativa* L. Rep. Kihara. Inst. Biol. Res. **2**, 42—53 (1952).

DARLINGTON, C. D.: Recent advances in cytology, 2nd Edit., pp. 510—511 and 559 to 560. Philadelphia: Blakiston 1937.

HENDERSON, M. T., B. P. YEH and B. EXNER: Further evidence of structural differentiation in the chromosomes as a cause of sterility in intervarietal hybrids of rice, *Oryza sativa* L. Cytologia (Tokyo) **24**, 415—422 (1959).

HSIEH, S., and H. OKA: Cytological studies of sterility in hybrids between distantly related varieties of rice, *Oryza sativa* L. Jap. J. Genet. **33**, 73—80 (1958).

KATO, S., H. KOSAKA and S. HARA: On the affinity of rice varieties as shown by fertility of hybrid plants. Rep. Bull. Sci. Fak. Terkult., Kyushu Imp. Univ., Japan **3**, 132—147 (1928).

KUANG, H. H.: Studies on rice cytology and genetics as well as breeding work in China. Agron. J. **43**, 387—397 (1951).

MASHIMA, I., and H. UCHIYAMADA: Studies on the breeding of fertile tetraploid plants of rice. Bull. Nat. Inst. Agric. Sci., Ser. D. **5**, 104—136 (1955).

MCCLINTOCK, B.: Association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Z. Zellforsch **19**, 191—237 (1933).

MELLO-SAMPAYO, T.: An inversion occuring in an F_1 hybrid between two strains of *Oryza sativa* L. Genetica iberica **4**, 43—46 (1952).

MIZUSHIMA, U., and A. KONDO: Fundamental studies on rice breeding through hybridization between Japanese and foreign varieties. II. Structural difference of chromosomes between a Japanese and Indian variety proved by anamolous mode of segregation in apiculus anthocyanin in their hybrid. Jap. J. Breed. **10**, 1—9 (1960).

OKA, H.: Gene analysis of intervarietal hybrid sterility and certation due to certain combinations of "gamete-development-genes" in rice. Jap. J. Breed. **3**, 23—30 (1953). — Studies on tetraploid rice. VI. Fertility variation and segregation ratios for several characters in tegraploid hybrids of rice, *Oryza sativa* L. Cytologia (Tokyo) **20**, 258—266 (1955a). — Change of gene frequency and a restriction on gene recombination in hybrid population of rice due to gamete development genes and duplicate fertility genes. J. Agric. Ass. China, N.S. **10**, 22—28 (1955b). — The polygenic nature of the gamete development genes controlling intervarietal hybrid sterility in rice. Jap. J. Breed. **6**, 51—55 (1956). — Gene analysis for the sterility of the hybrids between distantly related varieties of cultivated rice. J. Genet. **55**, 397—409 (1957).

RAO, P. K. M.: Msc. Unpublished thesis, I.A.R.I. New Delhi 1960.

SAMPATH, S., and H.K. MOHANTY: Cytology of semi-sterile rice hybrids. Curr. Sci. **23**, 182—183 (1954).

SHASTRY, S. V. S.: Embryological and cytological studies in the genus *Melilotus*. Ph. D. Thesis, University of Wisconsin 1958.

—, and R. N. MISRA: Pachytene analysis in *Oryza sativa* L. Sterility in *japonica-indica* rice hybrids. Curr. Sci. **30**, 70—71 (1961).

— D. R. RANGA RAO and R. N. MISRA: Pachytene analysis in Oryza. I. Chromosome morphology in *Oryza sativa* L. Indian J. Genet. Plant. Breed. **20**, 15—21 (1960).

— W. K. SMITH and D. C. COOPER: Species differentiation in *Melilotus*. Amer. J. Bot. **47**, 613—621 (1960).

STEBBINS jr., G. L.: Cytogenetic studies in *Paeonia*. II. The cytology of the diploid species and hybrids. Genetics **28**, 83—110 (1938). — Variation and evolution in plants. Columbia University Press 1950. — The inviability, weakness and sterility of interspecific hybrids. Advanc. Genet. **9**, 147—215 (1958).

—, and A. VAARAMA: Artificial and natural hybrids in the *Gramineae*, tribe *Hordeae*. Hybrids and allopolyploids between *Elymus glaucus* and *Sitanion* spp. Genetics **39**, 378—395 (1954).

STURTEVANT, A. H.: The effect of unequal crossing over at the *Bar* ocus in *Drosophila*. Genetics **10**, 117—147 (1925). — Essays on evolution. III. On the origin of interspecific sterility. Quart. Rev. Biol. **13**, 333—335 (1938).

—, and T. H. MORGAN: Reverse mutation of the *Bar* gene correlated with crossing over. Science **57**, 746—747 (1923).

SWAMINATHAN, M. S., and A. T. NATARAJAN: Cytological and genetic changes induced by vegetable oils in *Triticum*. J. Hered. **50**, 177—187 (1959).

TERAO, T., and U. MIZUSHIMA: Some considerations on the classification of *Oryza sativa* L. Jap. J. Bot. **10**, 213—258 (1939).

TING, Y. C.: Inversion and other characteristics of Teosinte chromosomes. Cytologia (Tokyo) **23**, 239—250 (1958).

VENKATSWAMY, T.: Genetical and cytological studies of sterility in inter-racial hybrigs of rice (*Oryza sytiva* L.). Assoc. I.A.R.I. Thesis (1957, unpublished).

WHITE, M. J. D.: Cytogenetics of orthopteroid insects. Advanc. Genet. **4**, 268—326 (1951).

YAO, Y., M. T. HENDERSON and N. E. JODON: Cryptic structural hybridity as a probable cause of sterility in intervarietal rice hybrids. Cytologia (Tokyo) **23**, 46—55 (1958).

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MEIOTIC BEHAVIOR OF AN UNEQUAL BIVALENT IN THE GRASSHOPPER CALLIPTAMUS PALAESTINENSIS BDHR.

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With 15 Figures in the Text

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The *Orthoptera* have been the classic source of unequal bivalents for meiotic studies and in most of the examples the extra segment of the longer member is heterochromatic. WHITE (1954) classified unequal bivalents into three types according to the mode of separation of the extra segment at the first meiotic division: (1) reductional, (2) equational, and (3) either reductional or equational. The third type of unequal bivalent has been reported only for the *Acrididae* where it has been found by different workers in at least eight species (WHITE 1954).

Previous workers encountered difficulties in attempting to explain how an acrocentric chromosome with an extra, terminal segment could separate reductionally at either first or second division. The unequal bivalents usually had but one chiasma and, at diplotene and diakinesis, showed only a cross configuration which was therefore accepted as the one leading not only to the equational but also to the reductional division at first anaphase. WENRICH (1916) accounted for the reductional division by assuming a centromere shift and his ideas were followed by MCCLUNG (1928) and CAROTHERS (1936). DARLINGTON (1937) and WHITE (1954) suggested that reductional division was a consequence of crossing over in the short arm, on the opposite side of the centromere from the extra segment; this explanation, however, was not confirmed by observations at metaphase I which failed for most species to show the configurations expected from crossing over in the short arm.

In 1952 SHARMAN described a seemingly different kind of unequal bivalent in *Exarna includens* (WALK.) in which the extra segment was interstitial. At diakinesis and diplotene he observed, in addition to well known cross configurations, another with a centrally located heterochromatic segment confined by euchromatin on both sides. This latter configuration was explained as the result of crossing over in the same arm as the extra segment but distal to it and would be expected to lead to the observed reductional separations at the first division.

The present study was made of an unequal bivalent in *Calliptamus palaestinensis* BDHR. in which the apparently terminal extra segment can be shown to be interstitial. It seems probable, therefore, that the previously puzzling cases may prove explicable in the same fashion.

Materials and Methods

Calliptamus palaestinensis BDHR. belongs to the subfamily *Catantopinae* of the family *Acrididae (Orthoptera)*. The animals were identified by Mr. M. P. PENER, Department of Zoology, The Hebrew University, Jerusalem, to whom the author is indebted. All individuals were collected during October and November, 1958 in different localities in Israel. For pachytene, diplotene, and diakinesis, the testes were squashed directly in iron acetocarmine but prior fixation in absolute alcohol, glacial acetic acid (3:1) was necessary for comparable results with metaphase I through anaphase II. Observations and photographs were made from either temporary or permanent preparations. All photographs of entire complements are reproduced at 1260×, of individual entities, at 1775×.

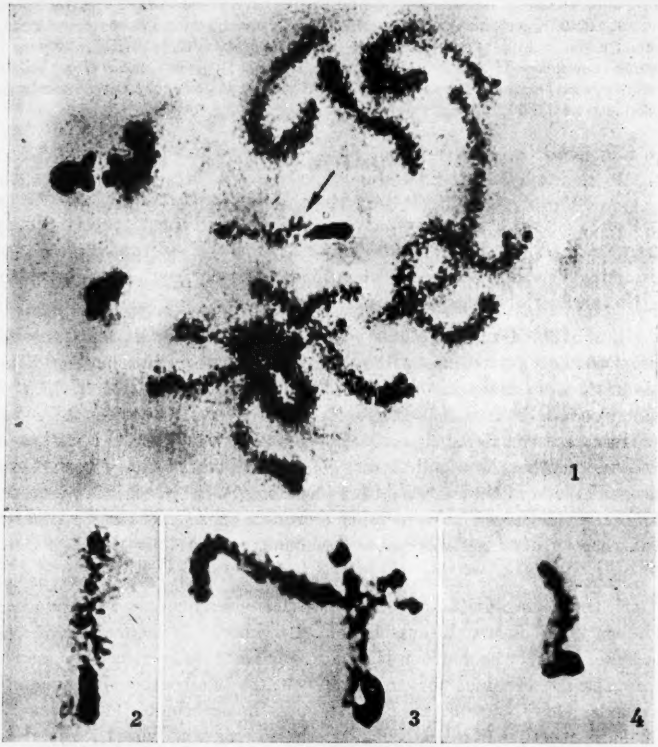
Observations

The males of *C. palaestinensis* have 23 chromosomes, 22 autosomes and one X; two of the eleven autosomal pairs are considerably smaller than the others. The unequal bivalent observed during meiosis was formed by the addition of a heterochromatic segment to one member of the smallest chromosome pair.

Unequal bivalents were found in two of the four populations sampled: in two of the twelve males from Ahihud (near Acre) and in ten of the 124 from Jerusalem but in none of the 15 from Ein Harod (Yizreel Valley) or the 19 from Beit Dagon (near Tel Aviv). Since the latter two localities are situated geographically between the other two, failure to find unequal bivalents in the samples from them may have been due to the small size of the samples. Individuals with heterochromatic supernumerary chromosomes were also present in the Jerusalem population and these will be described in a subsequent report.

Pachytene. The earliest analyzable stage was mid-pachytene at which the euchromatic and heterochromatic components of the unequal bivalent were clearly distinguishable. At this and subsequent stages the heterochromatic segment was about one-third as long as the heterochromatic X chromosome. In some cells, the heterochromatic segment extends singly beyond the paired euchromatic region (Fig. 1 and 2) while in others it is folded back, its tip now pairing with the distal end of the short homologue (Fig. 3 and 4). Among 89 cells at late pachytene in one individual, the heterochromatic segment was single in 25 cells and folded back with the ends paired in 44 cells; 20 cells were not analyzable in this regard. Pairing was thus observed in 64 per cent of the analyzable cells. In no instance was a fold back observed to the side opposite the euchromatic association. Thus, non-homologous association as originally described by MCCLINTOCK (1933) for heterochromatic B chromosomes in maize cannot be the sole factor responsible for the fold back of the extra segment. As can be seen in the Table not all these distal associations resulted later in chiasmata.

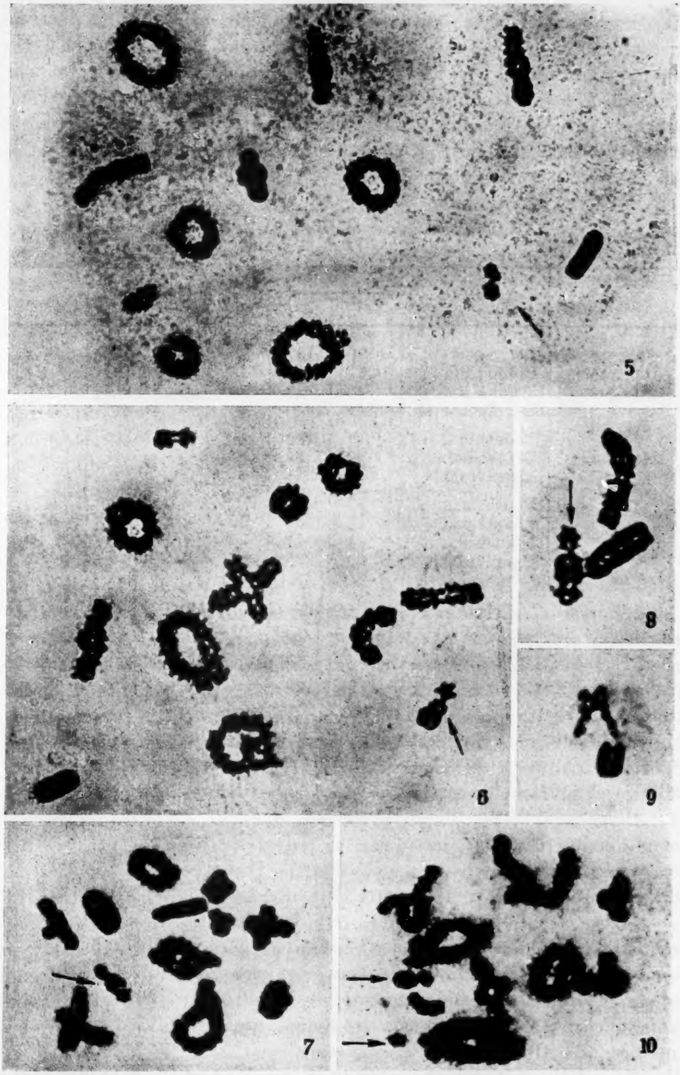
Diplotene. The single chiasma observed in the unequal bivalent was one of two types. In the majority of cases, there was a chiasma between the centromere and the unequal segment and the heteromorphic homologues lay parallel (Fig. 9). In the minority, a chiasma near the ends of



Figs. 1—4. Pachytene. 1: Unequal bivalent with ends unpaired (arrow); note split of heterochromatic segment. 2: Unequal bivalent of Fig. 1 enlarged. 3 and 4: The heterochromatic segment folded back; distal ends of the homologues paired

the homologues gave a configuration in which two euchromatic segments occurred on opposite sides of the heterochromatic.

Diakinesis. On their response to the contraction of diakinesis, the two types of configurations observed at diplotene became strikingly clear. The minority type acquired a "rolling pin" appearance with the thicker, heterochromatic segment between two thinner, euchromatic handles (Fig. 7 and 8). The majority type, with the chiasma between



Figs. 5—10. Diplotene and diakinesis. 5: Smallest bivalent normal. 6: Unequal bivalent, rod configuration. 7 and 8: Unequal bivalent, rolling pin configuration. 9: Unequal bivalent at diplotene; proximal chiasma; heterochromatic segment terminal and markedly split. 10: Unequal homologues as univalents

the centromere and the extra segment, did not open out into a cross configuration, but because of the localization of the chiasma near the centromere, formed a rod with the heterochromatic segment at one end (Fig. 6). The rod configuration was the one observed by previous workers. The frequencies of the two types of configurations as well as of the occasional failure of bivalent formation (Fig. 10) are cited in the Table.

Table. *Configurations of unequal homologues at spermatogenesis in C. palaestinensis*

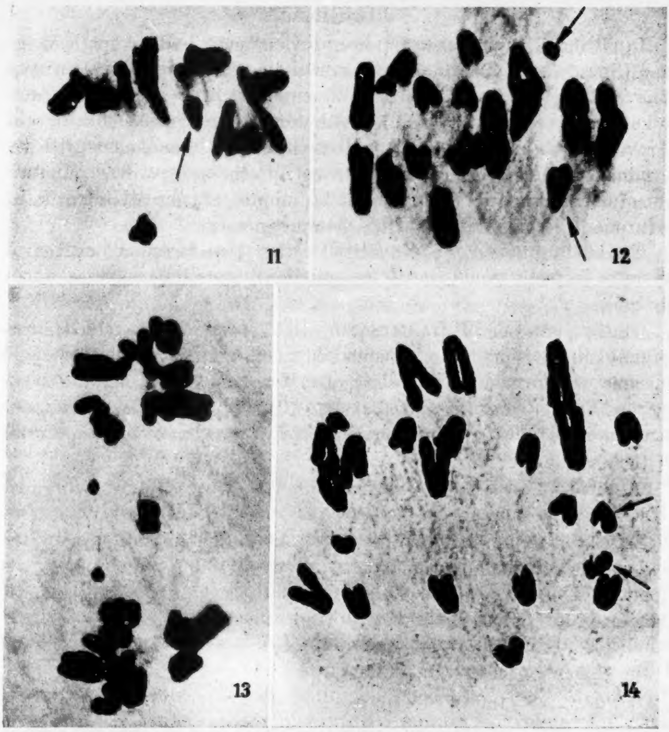
Stage	Configuration	Male No.				Total	% of analyzable
		806	832	846	8102		
Diplotene-Diakinesis	Rod.	193	147	39	—	379	90.2
	Rolling Pin . .	11	10	10	—	31	7.4
	Univalents. . .	10	0	0	—	10	2.4
	Unanalyzable .	14	0	0	—	14	—
Metaphase I	Bivalents . . .	19	49	—	33	101	96.2
	Univalents. . .	2	2	—	0	4	3.8
	Unanalyzable .	0	1	—	9	10	—
Anaphase I	Equational. . .	33	29	1	—	63	88.7
	Reductional . .	1	5	—	—	6	8.5
	Univalents. . .	1	1	—	—	2	2.8
	Unanalyzable .	0	3	—	—	3	—

Metaphase I. Further contraction of the chromosomes prevented a statistical analysis at this stage but bivalents of the two types were clearly identifiable in many instances. The rolling pin bivalents were parallel with the spindle axis, their euchromatic segments extending toward opposite poles (Fig. 11); the rod bivalents lay transversely. Univalents were again present in a small percentage of cells (Table). Both CAROTHERS (1931) and SHARMAN (1952) have observed occasional failure of bivalent formation by the unequal homologues.

Anaphase I. The majority of the unequal bivalents separated equationally (Fig. 14), the minority, reductionally (Fig. 12), in fairly exact conformity to the frequencies of the two types of configurations at diakinesis (Table). The univalents divided equationally (Fig. 13).

Second meiotic division. When the first meiotic division had been reductional, the second was equational with regards to the extra segment and vice versa. The univalents were not observed to divide again and apparently segregated independently at anaphase II. The sperms would consequently contain one of the two homologues of the unequal bivalent, neither, or both.

The relation between the position of chiasma and the behavior of the unequal bivalents at diakinesis and MI are summarized in Fig. 15.



Figs. 11—14. Metaphase I — anaphase I. 11 and 12: Reductional division of unequal bivalent. 13: Division of univalents of the unequal homologues. 14: Equational division of unequal bivalent

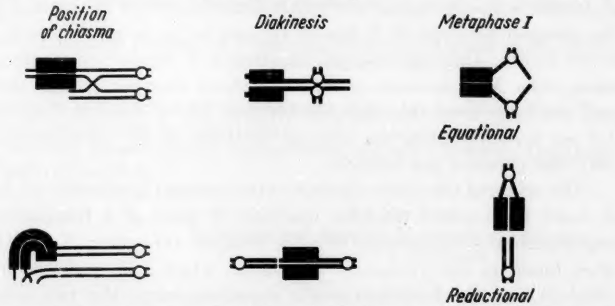


Fig. 15. Diagramatic representation of the stages leading to the equational and reductional division of the unequal bivalent at the first meiotic division

Discussion

In all the Orthopteran examples previously studied except *E. includens* (Sharman 1952), the extra segment in the unequal bivalent was believed to be terminal. In *C. palaestinensis* the extra segment appeared to be terminal (Fig. 1, 2 and 9) but the formation of chiasmata beyond it revealed the presence of terminal euchromatin, homologous with the terminal segment of the normal member. Pachytene pairing configurations further indicated the presence of a minute, not directly identifiable euchromatic segment beyond the heterochromatic.

The assumption of an interstitial rather than terminal location of the extra segment would clarify the previously puzzling cases (see introduction).

The explanation of Darlington (1937) and White (1954), that reductional division in the unequal bivalent occurred after the single chiasma was formed in the short arm, can apply only to the single species, *Stauroderus bicolor* (Darlington 1936) in which are to be found at metaphase I the configurations expected if crossing over had occurred in the short arm.

A bivalent with a chiasma in the short arm as for instance, the "Ditactic" chromosome of *Stethophyma gracile* (McClung 1928) gives an MI configuration in which the long arms of the chromosomes are free and parallel to the equatorial plate. While dividing reductionally, the unequal bivalents of all species except *Stauroderus bicolor* show that the long arms of the chromosomes are not free. They lie, instead, perpendicular to the equatorial plate at MI. This is what should be expected if the chiasma occurred in the long arm.

While the unequal bivalents of other species, particularly of *Phrynotettix tschivavensis* (Wenrich 1916), *Stethophyma gracile*, and *Trimerotropis citrina* (Carothers 1931) are very similar to those of *E. includens* (Sharman 1952) and *C. palaestinensis*, these all differ from that of *S. bicolor*; while the former are euchromatic except for the extra segment, the unequal bivalent of *S. bicolor* appears to be heterochromatic in its entire length. Only the unequal bivalent of *S. bicolor* has an observable short arm. As a result of its ability to form chiasma in both the short and the long arms this unequal bivalent has a chiasma frequency of 1.3 per bivalent, while the unequal bivalents of the other species have only one chiasma per bivalent.

The origin of the extra segment of the unequal bivalent is not known; it could have arisen from an insertion of most of a heterochromatic supernumerary chromosome into the smallest autosome. No individuals were found in the Jerusalem population which had both the unequal bivalent and the heterochromatic supernumerary; the two could not therefore be compared in the same animals, nor possible pairing observed.

Summary

Unequal bivalents were found in two of four populations of the short horned grasshopper, *Calliptamus palaestinensis* BDHR. sampled in Israel.

The inequality of the homologues was due to an extra segment which was heterochromatic and apparently terminal. Pairing configurations at pachytene and position of chiasmata at later stages revealed however that the extra segment was interstitial, and the long member terminated in a minute segment homologous to the terminal part of its normal partner. The percentage of reductional divisions at anaphase I corresponded well with the percentage of terminal chiasmata (i.e. distal to the extra segment) observed at diakinesis.

The assumption of an interstitial position of the extra segment would explain the previously puzzling examples of unequal bivalents in *Orthoptera* whose reductional divisions have not been readily accounted for otherwise.

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References

CAROTHERS, E. E.: The maturation divisions and segregation of heteromorphic homologous chromosomes in *Acrididae (Orthoptera)*. Biol. Bull. **61**, 324—349 (1931). — Components of the mitotic spindle with especial reference to the chromosomal and interzonal fibers in the *Acrididae*. Biol. Bull. **71**, 469—491 (1936).

DARLINGTON, C. D.: Crossing-over and its mechanical relationships in *Chorthippus* and *Stauroderus*. J. Genet. **33**, 465—500 (1936). — Recent Advances in Cytology, 2nd edit. London: Churchill; Philadelphia: Blakiston 1937.

MCCLINTOCK, B.: The association of non-homologous parts of chromosomes in the mid-prophase of *Zea mays*. Z. Zellforsch. **19**, 191—237 (1933).

MCCLUNG, C. E.: Differential chromosomes of *Mecostethus gracilis*. Z. Zellforsch. **7**, 756—778 (1928).

SHARMAN, G. B.: The cytology of Tasmanian short horned grasshoppers *(Orthoptera: Acridoidea)*. Pap. roy. Soc. Tasm. **86**, 107—122 (1952).

WENRICH, D. H.: The spermatogenesis of *Phrynotettix magnus*, with special reference to synapsis and the individuality of the chromosomes. Bull. Mus. Comp. Zool. Harv. **60**, 57—135 (1916).

WHITE, M. J. D.: Animal cytology and evolution, 2nd edit. Cambridge: Cambridge University Press 1954.

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TRISOMY FOR CHROMOSOME NO. 18 IN MAN*

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With 2 Figures in the Text

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Introduction

In the classification of human chromosomes by Patau (1960), the group E contains the chromosomes Nos. 16—18 and is identical with group 16—18 of the Denver system[1]. The distinction between the three pairs, especially between No. 17 and No. 18, is often difficult or impossible, but in very favorable mitoses it can be made unequivocally.

The first cases of trisomy for an E chromosome were independently and simultaneously announced by Edwards, Harnden, Cameron, Crosse and Wolff (1960), who described such a patient in detail, and by Patau, Smith, Therman, Inhorn and Wagner (1960), who briefly mentioned their discovery of two E trisomics. A full report on these two patients was published by Smith, Patau, Therman and Inhorn (1960). In an addendum they mentioned that they had found four more E trisomics, that all their six patients displayed the same syndrome, and that this trisomy syndrome seemed to be the same as that described by Edwards *et al.* (1960). However, whereas the British group identified the extra chromosome as No. 17, Smith *et al.* (1960) found it to be No. 18. It hardly needs saying that trisomy for different chromosomes cannot be expected to cause patterns of congenital anomalies that are similar enough to appear as one and the same clinical syndrome.

It is the purpose of the present communication, first, to show that the seven patients mentioned do indeed clinically represent the same syndrome, second, to attempt a definite identification of the extra chromosome responsible for this syndrome. The clinical aspects will here be touched upon only to the extent necessary for the first purpose; a full report on the four patients that have not yet been described will be published elsewhere.

* This investigation was supported by Grant C-3313 from the U.S. Public Health Service and Grant T-167 from the American Cancer Society, Inc.

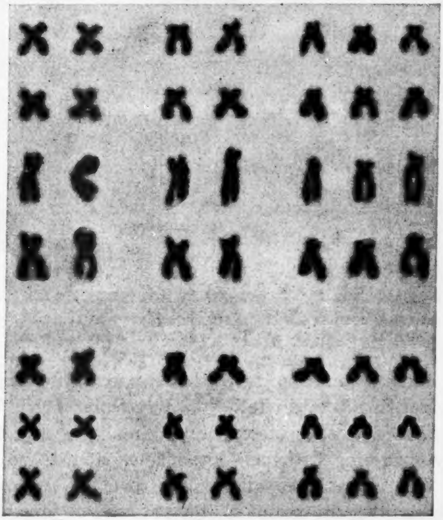
[1] "A proposed standard system of nomenclature of human mitotic chromosomes." Amer. J. Human Genet. 12, 384—388 (1960).

Material and methods

The original cytological work was done on bone marrow. In the beginning short-term cultures were used in the form described by PATAU *et al.* (1960). In an attempt to improve the rather low reliability of this procedure we abandoned culture altogether, injecting instead the infants subcutaneously with colcemide, a satisfactory dose being 0.15 mg per kg body weight. The bone marrow was aspirated after about five hours, diluted 1:10 with 1.12% sodium citrate, and fixed after about 30 minutes (incl. centrifugation) in 1:3 acetic alcohol. Semi-permanent Feulgen slides were made as described previously, acetic-orcein being added for more intense staining. This method appears to be more efficient than short-term culture, but its reliability leaves still much to be desired. It was abandoned in favor of tissue cultures. With these, the techniques described by THERMAN, PATAU, SMITH and DEMARS (1961), who followed closely the recommendations by TJIO and PUCK (1958), were used.

Cytological results

The bone marrow preparations from the first four patients ranged from poor to mediocre. In each case it could be established that in



16 17 18

Fig. 1. The E chromosomes incl. three No. 18 from four cells (top) of case 11 (tissue culture) and three cells (bottom) of case 41 (bone marrow). Feulgen, orcein. ×2740

addition to an apparently normal complement of 46 chromosomes there was an extra chromosome that belonged to the E group. A more precise identification of this chromosome was impossible. Bone marrow slides from the fifth patient contained mitoses that provided fairly good, but

not entirely conclusive, evidence that the extra chromosome was No. 18. In these mitoses there were definitely only two chromosomes No. 16. The bone marrow preparations of the sixth patient (case 41) were unusually good (see Fig. 3 by Smith *et al.* 1960) and yielded unequivocal evidence of the extra chromosome being No. 18 (Fig. 1).

Attempts to obtain tissue cultures of skin from the available E trisomics met for a while with failure, so much so that the suspicion arose

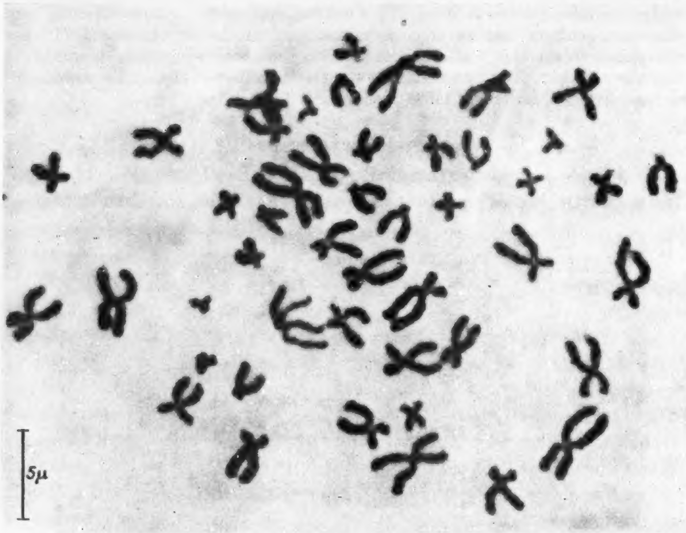


Fig. 2. The 47 chromosomes of case 11 (E chromosomes from this cell shown separately in Fig. 1, top line). Tissue culture. Feulgen, orcein

whether the extreme failure to thrive that is part of the syndrome might not have its basis in a lack of vitality on the cellular level. However, the difficulties may have been coincidental. Finally, a skin tissue culture from the last surviving E trisomic (case 11) started to grow and is now flourishing. The analysis of suitable cells revealed an entirely normal chromosome complement except for the presence of three instead of two chromosomes No. 18 (Figs. 1 and 2). The cytological findings are summed up in Table 1.

The No. 18 trisomy syndrome

For the present purpose it will suffice to list only the most regularly encountered and readily recognizable anomalies (Table 2). They add up to a well defined syndrome. It will be seen that the anomalies

reported by EDWARDS *et al.* (1960) for their E trisomic form a strikingly similar pattern, a much too similar pattern to be ascribed to trisomy for a non-homologous chromosome. There is even more agreement than the

Table 1. *Cytological results, near-diploid cells only*
For cases 8 and 19 see SMITH *et al.* (1960)

Patient	Material		Chromosome Number					Extra Chromosome identified as	Total
	Source	Method	≦45	46	47	48	≧49		
Case 11, ♀	bone marrow	short-term culture	—	—	4	—	—	E	4
	skin	tissue culture	—	—	14	—	—	No. 18	14
Case 27, ♂	bone marrow	colcemide	—	1	25	3	—	E	29
Case 34, ♀	bone marrow	colcemide	—	—	8	—	—	N. 18 (?)	8
Case 41, ♀	bone marrow	colcemide	—	1	22	—	—	No. 18	23
Total			—	2	73	3	—		78

table indicates. EDWARDS *et al.* (1960) found in their patient "hypermobility of the shoulders", a condition also noted in at least one of our patients. They report "webbing" between the second and third toe; we have found syndactyly of the same toes in two of these patients. The

Table 2. *The diagnostically most useful anomalies of the No. 18 trisomy syndrome (based on seven infants)*

Anomaly	Frequency of occurrence in No. 18 trisomics (Wisconsin cases)	Present in trisomic described by EDWARDS *et al.* (1960)
Apparent mental retardation	6/6	+
Hypertonicity (moderate)	6/6	?
Flexion of fingers with index finger overlapping 3rd	6/6	+
Ears low set and malformed	6/6	+
Small mandible	6/6	+
Failure to thrive	6/6	+
Dorsiflexion of big toes	4/4	?
Patent ductus arteriosus	5/6	+
Interventricular septal defect	5/6	+
Inguinal and/or umbilical hernia	4/6	—

"webbing of the neck" observed by the British authors might well be related to a condition displayed by at least two of our patients although this was not the webbing characteristic for TURNER's syndrome but rather a general looseness of the skin that was particularly noticeable in this area. The British authors do not mention a dorsiflexion of the big toes, but neither did we note this not very conspicuous condition in the

first two patients. When it was discovered to be a significant stigma in the remaining patients the first two had already died. The non-occurrence of hepatitis in our patients merely shows how right EDWARDS *et al.* (1960) were when they called its presence in their E trisomic "probably fortuitous".

Discussion

The findings presented fully support the contention of the British authors that "the constellation of ... abnormalities is consistent with the type of disorder to be expected from autosomal trisomy, and it seems likely that it may denote a clinical syndrome as specific as mongolism". Within the limits imposed by the difficulties of obtaining technically first-rate cytological material, No. 18 could be identified as the extra chromosome—with perfect assurance for two patients and on fairly good evidence for a third of our six E trisomics. Undoubtedly, the pattern of congenital anomalies displayed by these six patients can properly be called the "No. 18 trisomy syndrome". If it is accepted that on clinical grounds this syndrome must also be attributed to the infant described by EDWARDS *et al.* (1960), it would seem that in this case, too, the responsible chromosome was No. 18 rather than No. 17 as claimed by these authors.

When we consider the possibility of a mistaken identification of the extra chromosome by the British authors we want to make very clear that this should not be construed as a reflection upon the cytological quality of these authors' work. In the best of slides there are very few mitoses in which the precise identity of an extra E chromosome can definitely be established. All too often it can be interpreted equally well as No. 17 or as No. 18. (On the other hand, we have never seen a mitosis from any of our patients in which it appeared not at least possible that the extra chromosome was No. 18.) The interpretation of their Fig. 4 by EDWARDS *et al.* (1960) was highly plausible, but we do not think that their identification of three chromosomes as No. 17 is entirely beyond question. This figure has evidently been printed to render the chromosomes darker than they appear in the microscope, which may lead to some exaggeration of the apparent mass of a short arm if this should happen to be relatively elongated (in the case of our Fig. 1 the contrast has been kept low so as to reproduce as closely as possible the microscopic appearance of the chromosomes). However, we are by no means certain that EDWARDS *et al.* (1960) were mistaken when they failed to recognize three chromosomes No. 18.

Our conviction that their patient represented the No. 18 trisomy syndrome, and therefore was not trisomic for No. 17, does not imply that the extra chromosome was a normal chromosome No. 18. A small

pericentric inversion could cause the latter to resemble No. 17, as could a suitable translocation involving the short arm of No. 18. These possibilities are not as farfetched as they might seem since structural heterozygosity in one of the parents might have brought about non-disjunction in the first place and thus caused the presence of an extra chromosome in the child. The resulting chromosome complement could not strictly be called trisomic even though the phenotypic effect might be the same or very nearly the same as that of pure trisomy for No. 18. Without further elaboration it may be pointed out that structural heterozygosity in one of the parents might also account for the reported occurrence of two miscarriages in addition to only one pregnancy with a normal child. In this context the relatively young age (31) of the mother is also of interest. Primary non-disjunction is much more frequent in older mothers as may be concluded from the age correlation not only in mongolism but also in our sample of No. 18 trisomics whose mothers had at conception the following age distribution: 46, 46, 39, 37, 29, 20. In the case of our own results the possibility of chromosomal rearrangements need not be considered, as our identification of the extra chromosome as No. 18 rests upon two, or even three patients.

Summary

A brief description of six cases of trisomy for an E chromosome is given and compared with the E trisomic reported by Edwards *et al.* (1960). All seven patients clearly represent the same syndrome. It is caused by trisomy for the chromosome No. 18.

References

Edwards, J. H., D. G. Harnden, A. H. Cameron, V. M. Crosse and O. H. Wolff: A new trisomic syndrome. Lancet **1960 I**, 787—790.

Patau, K.: The identification of individual chromosomes, especially in man. Amer. J. hum. Genet. **12**, 250—276 (1960).

— D. W. Smith, E. Therman, S. L. Inhorn and H. P. Wagner: Multiple congenital anomaly caused by an extra autosome. Lancet **1960 I**, 790—793.

Smith, D. W., K. Patau, E. Therman and S. L. Inhorn: A new autosomal trisomy syndrome: two cases of multiple congenital anomaly caused by an extra chromosome. J. Pediat. **57**, 338—345 (1960).

Therman, E., K. Patau, D. W. Smith and R. I. DeMars: The D trisomy syndrome and XO gonadal dysgenesis in two sisters. Amer. J. hum. Genet. (1961, in press).

Tjio, J. H., and T. T. Puck: Genetics of somatic mammalian cells. II. Chromosomal constitution of cells in tissue culture. J. exp. Med. **108**, 259—268 (1958).

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UNTERSUCHUNGEN ÜBER DIE VERTEILUNG DER BRUCHSTELLEN NATÜRLICHER UND STRAHLENINDUZIERTER CHROMOSOMENDISLOKATIONEN BEI DROSOPHILA SUBOBSCURA COLL.

Von

E. Kunze-Mühl

Mit 1 Textabbildung

(Eingegangen am 31. Dezember 1960)

Einleitung

Die Beobachtung, daß zahlreiche in den natürlichen Populationen von *Drosophila subobscura* vorkommende Inversionen gemeinsame Bruchenden haben, gab die Anregung zu den in der vorliegenden Mitteilung beschriebenen Untersuchungen. Es wurde von Kunze-Mühl und Sperlich (1955) und Kunze-Mühl und Müller (1958) versucht, zur Deutung dieses Phänomens verschiedene Hypothesen aufzustellen. Um der Verifizierung einer dieser Hypothesen näherzukommen, sollte untersucht werden, ob bei Röntgenbestrahlung bestimmte Stellen des Chromosomensatzes leichter brechen und disloziert verheilen als andere oder ob die Verteilung der bei einer cytologischen Analyse erfaßbaren röntgeninduzierten Bruchstellen eine zufällige ist.

Da alle Untersuchungen mit möglichst großer Genauigkeit durchgeführt werden sollten, war es notwendig, die bisher zu allen cytologischen Analysen an *Drosophila subobscura* verwendete Chromosomenkarte von Mainx, Koske und Smital (1953) durch eine detailreichere zu ersetzen, in der so weit als möglich alle, auch die feinsten Querscheiben eingezeichnet sind (Kunze-Mühl und Müller 1958). An Hand der neuen Karte wurden alle Bruchenden der in natürlichen Populationen bisher beobachteten Inversionen genau lokalisiert, wodurch sich für die Lage und die Koinzidenz von Bruchstellen mehrere Änderungen ergaben. Da außerdem auch einige erst in den letzten Jahren gefundene Inversionen eingetragen wurden, änderte sich das Bild der Bruchstellenkoinzidenzen gegenüber den Angaben von Kunze-Mühl und Sperlich (1955) sehr wesentlich. Es konnte jedoch die Vermutung bestätigt werden, daß die Verteilung der natürlichen Bruchstellen nicht zufällig ist. Um festzustellen, ob diese Annahme zu Recht besteht, sollte diese Verteilung mittels statistischer Berechnungen getestet und im Vergleich dazu auch die Verteilung der strahleninduzierten Bruchstellen statistisch geprüft werden.

Cytologische Analysen von strahleninduzierten Dislokationen wurden mehrfach an *Drosophila melanogaster* durchgeführt. BAUER, DEMEREC und KAUFMANN (1938), BAUER (1939), CATCHESIDE (1938) und KAUFMANN (1939) untersuchten vor allem Art und Frequenz des Auftretens von Dislokationen in Beziehung zur Strahlungsdosis. Auch HELFER (1941) und KOLLER und AHMED (1942), die mit *Drosophila pseudoobscura* arbeiteten, beschäftigten sich vorwiegend mit ähnlichen Problemen. Da in der vorliegenden Arbeit kein Wert auf die Überprüfung dieser Fragen bei *Drosophila subobscura* gelegt wurde, ist ein Vergleich mit den oben angeführten Untersuchungen nur teilweise möglich. Um die Ergebnisse dieser Arbeit aber so weit als möglich mit den Beobachtungen an *Drosophila pseudoobscura*, die ebenso wie *Drosophila subobscura* der *obscura*-Gruppe angehört, vergleichen zu können, wurden ähnliche Versuchsbedingungen gewählt, wie sie von KOLLER und AHMED (1942) beschrieben wurden.

Herrn Prof. Dr. F. MAINX danke ich für die Anregung zu dieser Arbeit, Herrn Prof. Dr. J. PFANZAGL und Herrn Dr. J. ROPPERT für ihre wertvolle Hilfe bei den statistischen Berechnungen. Für die Mithilfe bei der experimentellen Arbeit danke ich Frau Dr. EVA MÜLLER.

Material und Methoden

Die cytologische Analyse der röntgeninduzierten Dislokationen wurde an den Speicheldrüsenchromosomen der F_1-Larven aus den Kreuzungen bestrahlter Männchen mit unbestrahlten, unbegatteten Weibchen des Stammes „Küsnacht" vorgenommen. Die homozygote Struktur des Stammes „Küsnacht" wurde allen bisherigen Untersuchungen an *Drosophila subobscura* als Standardanordnung zugrunde gelegt. 10 Tage alte Männchen dieses Stammes wurden mit einem Siemens Monopan, 4 mA, 60 kV, Tubus 1,5 cm bestrahlt. In der Ebene der flachen Bestrahlungskammern, in denen die Fliegen während der Bestrahlung eingeschlossen waren, war die Leistung 1200 r pro Minute. Die mit 5000 r bestrahlten Männchen wurden mit 10 Tage alten unbestrahlten, unbegatteten Weibchen zur Kopula gebracht. Nach 2 Tagen wurden die Tiere in neue Gläser umgesetzt, in diesen 3 Tage gelassen und hierauf die Männchen aus den Gläsern entfernt. Die begatteten Weibchen wurden während der nächsten 3 Tage mehrmals in neue Gläser umgesetzt, und die mit Eiern beschickten Flaschen wurden sodann zur Präparation vorbereitet. Die Verwendung von 10 Tage alten Männchen, die sofort nach dem Schlüpfen isoliert worden waren, bietet die Gewähr, daß ein großer Vorrat reifer Spermien zur Bestrahlung gelangt. Die Weibchen sind im Alter von 10 Tagen besonders kopulationsbereit, so daß bald nach dem Ansetzen der Kreuzungen mit Kopula und Eiablage gerechnet werden kann. Durch das Entfernen der Männchen aus den Kulturen nach 5 Tagen sollte gewährleistet werden, daß nur die zum Zeitpunkt der Bestrahlung bereits voll ausgereiften Spermien zur Befruchtung gelangen. Die optimale Entwicklung der Larven sollte durch das wiederholte Umsetzen der Imagines, das einer Übervölkerung der Kulturen vorbeugt, gesichert werden.

Es wurden 5 Bestrahlungen durchgeführt und insgesamt 646 F_1-Larven untersucht. Die Färbung der Speicheldrüsen erfolgte in 2% Orcein mit 60% Essigsäure. Die erste Beurteilung wurde an Frischpräparaten vorgenommen und alle

Präparate, in denen Dislokationen festgestellt wurden, wurden als Dauerpräparate in Euparal eingeschlossen, um sie später genau analysieren zu können. Die Lokalisation der Dislokationen und ihrer Bruchstellen erfolgte an Hand der von KUNZE-MÜHL und MÜLLER (1958) veröffentlichten Chromosomenkarte.

Ergebnisse

1. Mutationshäufigkeit

Von den 646 F_1-Larven wiesen nach Bestrahlung mit 5000 r 92 Tiere Dislokationen auf, das sind $14{,}24 \pm 1{,}37\%$. EIGNER (1952) fand bei ähnlichen Versuchen an *Drosophila subobscura* einen noch geringeren Prozentsatz veränderter Spermien. Die Ergebnisse beider Untersuchungen zeigen eine starke Abweichung von den Ergebnissen von HELFER (1941) und KOLLER und AHMED (1942) bei *Drosophila pseudoobscura* sowie auch von den Resultaten, die BAUER, DEMEREC und KAUFMANN (1938) und BAUER (1939) bei *Drosophila melanogaster* erhielten. Auch FIALA und NEUBERT (1951) fanden nach Bestrahlung von *Drosophila hydei* von den unseren abweichende Werte. Von HELFER wurden bei Bestralung von *Drosophila pseudoobscura* mit 5000 r 32% veränderter Spermien angegeben. Der von KOLLER und AHMED angegebene Prozentsatz liegt trotz der verwendeten niedrigeren Bestrahlungsdosis von 4500 r sogar noch höher und zwar bei $40{,}0 \pm 2{,}23\%$. *Drosophila melanogaster* wies bei Bestrahlung mit 5000 r $40{,}0 \pm 3{,}33\%$ bzw. $44{,}9 \pm 2{,}02\%$ auf. FIALA und NEUBERT berechneten für *Drosophila hydei* eine Rate von $27{,}8 \pm 4{,}6\%$ für 4800 r. Wenn dieser starke Unterschied zwischen den Prozentsätzen veränderter Spermien bei *Drosophila subobscura* einerseits und *Drosophila pseudoobscura*, *Drosophila melanogaster* und *Drosophila hydei* andererseits auf eine verschieden hohe Bruchanfälligkeit der Chromosomen oder auf Unterschiede in der Bruchheilungsfrequenz zurückzuführen wäre, müßte man annehmen, daß in den durch Röntgenstrahlen veränderten Spermien von *Drosophila subobscura* auch durchschnittlich weniger Chromosomenbrüche auftreten als bei anderen *Drosophila*-Arten. Dies ist aber kaum der Fall. Bei *Drosophila subobscura* fanden sich in 92 veränderten Spermien insgesamt 234 Chromosomenbrüche, die disloziert verheilt waren, das entspricht einem Durchschnitt von 2,54 Bruchstellen pro Spermium. Bei *Drosophila pseudoobscura* liegt der entsprechende Wert bei 2,63, bei *Drosophila melanogaster* bei 3,13 bzw. bei 3,21.

Es wurden in dem untersuchten Material in 61 Präparaten Translokationen und in 17 Präparaten Inversionen gefunden. In 13 Präparaten waren sowohl Inversionen als auch Translokationen vorhanden. Das Verhältnis zwischen Translokationen und Inversionen beträgt ungefähr 2:1 und entspricht somit den Resultaten bei *Drosophila pseudoobscura*, *Drosophila melanogaster* und *Drosophila hydei*. Außerdem wurde

in einem Präparat ein endständiges deficiency gefunden. Die Aussicht, daß eine derartige Dislokation erhalten bleibt und zur Beobachtung gelangt, scheint sehr gering zu sein, da nur sehr wenige ähnliche Beobachtungen bei anderen *Drosophila*-Arten beschrieben sind. Das endständige deficiency ist durch ein Einbruchereignis entstanden. Der Großteil der veränderten Spermien wurde von Zweibruchereignissen betroffen. Die höchste beobachtete Anzahl von Brüchen, die in einem Spermium stattgefunden hatten, war 7. In Tabelle 1 ist eine Übersicht

Tabelle 1. *Verteilung der Bruchstellen in bestrahlten Spermien*

	Anzahl der Brüche								Gesamt
	0	1	2	3	4	5	6	7	
Zahl der Spermien	554	1	64	12	10	2	2	1	646

über die Anzahl der Spermien mit 0—7 Bruchstellen gegeben. Untersuchungen von HELFER (1941) und KOLLER und AHMED (1942) an *Drosophila pseudoobscura* und von BAUER, DEMEREC und KAUFMANN (1938) an *Drosophila melanogaster* zeigten, daß die gerade Anzahl von Bruchstellen pro Spermium relativ häufiger war als die ungerade. Wie aus Tabelle 1 ersichtlich ist, konnte diese Erscheinung bei *Drosophila subobscura* nicht eindeutig beobachtet werden.

2. *Verteilung der Bruchstellen auf die Chromosomen*

Im folgenden soll die Verteilung der natürlichen und die der röntgeninduzierten Bruchstellen auf die Chromosomen vergleichend betrachtet werden. In den natürlichen Populationen von *Drosophila subobscura* konnten bisher insgesamt 39 Inversionen beobachtet werden, die durch 78 Chromosomenbrüche entstanden sind. Die genaue Lokalisation ihrer Bruchenden erfolgte an Hand der von KUNZE-MÜHL und MÜLLER (1958) veröffentlichten Chromosomenkarte, wobei die beiden Brüche der von STUMM-ZOLLINGER (1952) gesehenen Inversion $O_{(21)}$ nicht genau festgestellt werden konnten. Es wurden aus diesem Grund nur die übrigen 76 Chromosomenbrüche für alle folgenden Berechnungen verwendet. Bei der cytologischen Analyse der strahleninduzierten Bruchstellen wurden insgesamt 234 Bruchstellen gezählt. Alle Bruchstellen, die an derselben Chromosomenstelle oder zumindest sehr nahe beieinander zu liegen schienen, wurden mehrmals genau geprüft, um alle Koinzidenzen möglichst genau zu erfassen. Es sollen im folgenden Bruchstellen dann als identisch oder als Koinzidenz bezeichnet werden, wenn sie in dem Raum zwischen zwei benachbarten Querscheiben liegen, so wie dies KUNZE-MÜHL und MÜLLER (1958) auch für die Bruchstellen natürlicher Inversionen durchgeführt haben. Im Falle der röntgeninduzierten

Bruchstellen kann, im Gegensatz zu einem Teil der Spontanbruchstellen, bei allen identischen Bruchstellen von „echten" Bruchstellenkoinzidenzen gesprochen werden, da von wiederholten Brüchen an einer bestimmten Chromosomenstelle immer wieder dieselben, auf der Chromosomenkarte benachbart liegenden, Bruchenden betroffen werden. Es ist also in diesem Fall nicht notwendig beide Bruchenden eines Bruches auf der Chromosomenkarte einzutragen, wie das für alle beobachteten Spontanbrüche durchgeführt werden mußte. In dem vorgelegten Schema (Abb. 1) sind die 5 langen Chromosomen von *Drosophila subobscura* durch 5 waagrechte Linien symbolisiert, auf denen alle beobachteten Bruchstellen mit senkrechten Strichen eingetragen wurden. Oberhalb der waagrechten Linien sind die natürlichen Bruchstellen eingetragen, wobei wie auf der Chromosomenkarte die jeweiligen Bruchenden jeder Bruchstelle mit der Nummernbezeichnung der jeweiligen Inversion bezeichnet sind. Unterhalb der Linien sind alle röntgeninduzierten Bruchstellen ohne Bezeichnung eingetragen.

Für die Frage der Verteilung der Bruchstellen auf die einzelnen Chromosomen müssen zunächst die Längenverhältnisse der Chromosomen berücksichtigt werden. Die relativen Längen der Chromosomen in reifen Speicheldrüsenkernen verhalten sich wie A:I:U:E:O = 1:1,20: 1,20:1,12:1,43. Dementsprechend müßten bei zufälliger Verteilung die 76 natürlichen und die 234 röntgeninduzierten Bruchstellen wie 1:1,20: 1,20:1,12:1,43 auf die Chromosomen A, I, U, E und O verteilt sein. Bei diesen und allen folgenden Berechnungen wurden im Ansatz die Zahlen nur bis zu einer Genauigkeit von 2 Dezimalen ermittelt. Die sich dadurch ergebenden geringen Abweichungen spielen für die Endresultate keine Rolle. In Tabelle 2 sind sowohl für die natürlichen

Tabelle 2. *Verteilung natürlicher und röntgeninduzierter Bruchstellen auf die Chromosomen A, I, U, E und O*

Chromosom	Natürliche Bruchstellen			Röntgeninduzierte Bruchstellen		
	beobachtet	erwartet	χ^2	beobachtet	erwartet	χ^2
A	4	12,57	5,84	(29)	—	—
I	6	15,09	5,48	55	49,57	0,59
U	16	15,12	0,08	47	49,68	0,45
E	14	14,04	0,00	38	46,12	1,43
O	36	17,96	18,12	65	59,00	0,61
Gesamt	76		29,52 $p < 0{,}001$	205 (234)		3,08 $p = 0{,}10$—$0{,}05$

wie für die durch Röntgenstrahlen erzeugten Bruchstellen die erwarteten den beobachteten Werten gegenübergestellt und die χ-Quadrate für die einzelnen Chromosomen sowie die Summen-χ-Quadrate und die

entsprechenden p-Werte angegeben. Im Falle der röntgeninduzierten Bruchstellen wurde das Geschlechtschromosom (A-Chromosom) nicht in die Berechnung mit einbezogen, da in diesem Chromosom nur ungefähr die Hälfte der Bruchstellen erfaßt werden konnte und daher ein Vergleich mit den Autosomen nicht zulässig ist. Für die natürlichen Bruchstellen wurde ein p von unter 0,001 errechnet. Dieses hoch signifikante Resultat hat, wie aus Tabelle 2 ersichtlich ist, seine Ursache offenbar in einer Anhäufung von Bruchstellen im O-Chromosom, während das A- und I-Chromosom offensichtlich wesentlich weniger Bruchstellen aufweisen, als zu erwarten wäre. Im Gegensatz dazu liegt das p für die röntgeninduzierten Bruchstellen zwischen 0,10 und 0,05, d.h., daß für alle 4 Autosomen eine ihrer relativen Länge entsprechende gleichmäßige Verteilung der Bruchstellen angenommen werden kann.

Es erschien nun von Interesse, die beiden unterschiedlichen Verteilungen der Bruchstellen auf die Chromosomen auch mit dem Heterogenitätstest zu prüfen. Bei dieser Berechnung mußte das A-Chromosom unberücksichtigt gelassen werden. Es wurden für die natürlichen und für die röntgeninduzierten Bruchstellen Mittelwerte der erwarteten Häufigkeit für jedes Chromosom berechnet und die Abweichungen der Beobachtungswerte mit der χ^2-Methode getestet (MATHER 1946). Das auf diese Weise erhaltene χ-Quadrat von 13,20 entspricht bei 3 Freiheitsgraden einem p von 0,01 — 0,001. Es zeigt sich also, wie zu erwarten war, eine deutliche Heterogenität der Daten und somit keine Übereinstimmung zwischen den beiden Verteilungen.

Ob allerdings bestimmte Bereiche innerhalb der Chromosomen mehr Bruchstellen aufweisen als andere, kann aus den oben angeführten Berechnungen noch nicht ersehen werden. So könnten z. B. die chromozentrumnahen Bereiche jedes Chromosoms eine höhere oder niedrigere Bruchstellenanzahl zeigen als die übrigen Chromosomenabschnitte oder ein nahe dem distalen Ende jedes Chromosoms gelegener Teil könnte eine von anderen Abschnitten abweichende Anzahl von Bruchstellen aufweisen. Allerdings müßte in solchen Fällen jedes Chromosom annähernd gleich große Bereiche besitzen, in denen eine ebenfalls annähernd gleich große Steigerung oder Senkung der Bruchstellenfrequenz vorliegen müßte. Eine Zufallsverteilung der Bruchstellen würde sich nach obiger Berechnung auch ergeben, wenn nur kleinere Bereiche oder auch nur bestimmte Punkte mehr Bruchstellen zeigen würden als andere, diese aber annähernd gleichmäßig über den ganzen Chromosomensatz verteilt wären. Der oben beschriebene Test sagt also über die Verteilung der Bruchstellen entlang den Chromosomen und somit über eine besonders hohe oder besonders niedrige Bruchstellenfrequenz in bestimmten kleineren Bereichen, als die ganzen Chromosomen es sind,

nichts aus. Es sollte nun durch verschiedene Testverfahren ermittelt werden, ob solche Stellen im Chromosomensatz von *Drosophila subobscura* im Falle der natürlichen und röntgeninduzierten Bruchstellen vorhanden sind und ob die Lage solcher Bereiche in jedem Chromosom dieselbe ist oder nicht.

3. *Verteilung der Bruchstellen auf die Chromosomenabschnitte*

a) Verteilung der natürlichen Bruchstellen

Wenn wir uns zunächst der Verteilung der 76 natürlichen Bruchstellen zuwenden, deren Frequenz schon in den einzelnen Chromosomen sehr unterschiedlich war, so fallen bei Betrachtung des Schemas die vielen Bruchendenkoinzidenzen auf. Auch andere Unregelmäßigkeiten in der Bruchverteilung lassen vermuten, daß die Verteilung der Bruchstellen innerhalb der Chromosomen keine zufällige ist. Wie schon von Kunze-Mühl und Müller (1958) begründet wurde, ist es notwendig, jeden Bruch einer Inversion durch zwei Bruchenden zu charakterisieren. Wo ein Bruch an einer Stelle eintritt, an die bereits durch ein vorangegangenes Inversionsgeschehen ein Bruchende verlagert worden ist, das auf der Standardkarte nicht neben dem durch diese Inversion nicht verlagerten Bruchende liegt, können auf der Standardkarte nicht zwei verschiedene Bruchstellen, sondern nur drei verschiedene Bruchenden eingetragen werden. In die Berechnung müssen in diesem Fall 1,5 Bruchstellen eingehen. Es wurde für jede der folgenden Berechnungen also nicht die Zahl der Bruchstellen, sondern die der Bruchenden pro Abschnitt ermittelt und die Hälfte dieser Zahl zur Berechnung der Verteilung der Bruchstellen verwendet, ohne Rücksicht auf die jeweils zu einer Bruchstelle gehörenden Bruchenden.

Da bei Betrachtung des Schemas auffällt, daß in den proximalen Teilen der Autosomen offensichtlich weniger natürliche Bruchstellen zu liegen scheinen als in den anderen Chromosomenabschnitten, sollte mittels der einfachen χ^2-Methode festgestellt werden, ob durch diese geringere Bruchfrequenz in den chromozentrumnahen Teilen eine gesicherte Abweichung von der Gleichverteilung bedingt wird. Jedes Chromosom wurde zunächst in 4 gleich lange Abschnitte geteilt und die Beobachtungswerte für jeden dieser Abschnitte ermittelt. Ein Vergleich mit den Erwartungswerten war nur für den gesamten Chromosomensatz mit Ausnahme des dot-Chromosoms möglich, da ein für jedes Chromosom gesonderter χ^2-Test wegen der zu kleinen Erwartungswerte nicht zulässig ist. Es wurden 2 Berechnungen durchgeführt, und zwar wurde geprüft, ob die Bruchstellenverteilung auf Viertelabschnitte der 5 langen Chromosomen mit der Annahme einer gleichmäßigen Verteilung auf alle Chromosomenteile übereinstimmt, und

zweitens wurde die Übereinstimmung mit der Zufallsverteilung unter Ausschluß der proximalen Viertel getestet (Tabelle 3).

Tabelle 3. *Frequenz natürlicher Bruchstellen in Viertelabschnitten gleicher Lage*

	Chromosom	Beobachtete Anzahl der Bruchstellen im				Gesamtzahl der Bruchstellen	χ^2	p
		1. Viertel prox.	2. Viertel	3. Viertel	4. Viertel			
	A	1	2	1	0	4		
	I	0	5	1	0	6		
	U	2,5	4	2,5	7	16		
	E	0	6,5	5,5	2	14		
	O	2	11	13	10	36		
I.	A—O	5,5	28,5	23	19	76	15,18	0,01—0,001
II.	A—O	—	28,5	23	19	70,5	1,93	0,50—0,30

Es zeigt sich also für die Berechnungen auf alle Chromosomenviertel eine signifikante Abweichung von der Zufallsverteilung, wogegen sich eine Übereinstimmung mit der Zufallsverteilung ergibt, wenn man bei der Berechnung die proximalen Viertel außer acht läßt. Damit ist bewiesen, daß die Abweichung von der Gleichverteilung natürlicher Bruchstellen auf Viertelabschnitte der Chromosomen auf der geringen Bruchstellenfrequenz in den proximalen Vierteln beruht, während die anderen Chromosomenabschnitte eine ungefähr gleich hohe Bruchstellenzahl aufweisen. Um festzustellen, ob die proximalen Teile, in denen die Bruchstellenfrequenz besonders niedrig ist, noch kleiner sind als Viertelabschnitte, wurden ähnliche Berechnungen auch für die Verteilung der Bruchstellen in Zehntelabschnitten der 5 langen Chromosomen durchgeführt (Tabelle 4).

Tabelle 4. *Frequenz natürlicher Bruchstellen in Zehntelabschnitten gleicher Lage*

	Chromosom	Beobachtete Anzahl der Bruchstellen im									Gesamtzahl der Bruchstellen	χ^2	p
		1. Zehntel prox.	2. Zehntel	3. Zehntel	4. Zehntel	5. Zehntel	6. Zehntel	7. Zehntel	8. Zehntel	9. Zehntel	10. Zehntel			
	A	1	0	0	1	1	0	1	0	0	0	4		
	I	0	0	2	0	3	0	1	0	0	0	6		
	U	0	0	3,5	0	3	1,5	0	2	4	2	16		
	E	0	0	2,5	2	2	2	2,5	2	1	0	14		
	O	1	1	1	5	5	5	8	3	3	4	36		
I.	A—O	2	1	9	8	14	8,5	12,5	7	8	6	76	19,21	0,05—0,02
II.	A—O	—	—	9	8	14	8,5	12,5	7	8	6	73	5,75	0,70—0,50

Auch hier mußte auf eine gesonderte Berechnung für die einzelnen Chromosomen verzichtet werden, weil die Erwartungswerte zu gering

sind. Für die zweite ebenfalls in Tabelle 4 angeführte Berechnung wurden die beiden proximalen Zehntel jedes Chromosoms außer acht gelassen. Bei der ersten Berechnung ergab sich ähnlich wie in Tabelle 3 ein allerdings etwas weniger signifikanter p-Wert für die Abweichung von der Gleichverteilung. Die zweite Berechnung ergab, daß für diese Abweichung die geringe Bruchstellenfrequenz in den beiden proximalen Zehntelabschnitten jedes Chromosoms ausschlaggebend sein muß, da in den übrigen Zehntelabschnitten die Übereinstimmung mit der Gleichverteilung wahrscheinlich erscheint.

Bei allen soeben beschriebenen Berechnungen wurden, wie z. B. aus Tabelle 3 ersichtlich ist, alle 1. (proximalen), alle 2. und alle 3. und 4. (distalen) Viertel aller 5 langen Chromosomen zusammengefaßt und die Bruchstellenfrequenz in diesen Abschnitten mit der χ^2-Methode getestet. Nimmt man nun an, daß eine besonders hohe oder eine besonders niedere Bruchstellenfrequenz in Abschnitten auftritt, die sich in den verschiedenen Chromosomen nicht in gleicher Lage befinden, so würde eine dadurch bedingte Abweichung von der Zufallsverteilung mittels der oben beschriebenen Methode nicht aufgedeckt werden. Vor allem aber würden überzufällig gehäufte Bruchstellenkoinzidenzen durch diesen Test nur dann erfaßt werden, wenn der Großteil dieser Koinzidenzen in bestimmten Abschnitten liegen würde, die in jedem Chromosom die gleiche Lage hätten. Da bei Betrachtung des Schemas eine solche Annahme durchaus nicht berechtigt erscheint, wurde zur Aufdeckung von vielleicht unregelmäßig über den ganzen Chromosomensatz verteilten Abschnitten mit höherer Bruchstellenfrequenz ein anderes Testverfahren verwendet. Als Zufallsverteilung wird die Poissonsche Verteilung angenommen, wobei die Erwartungswerte mit der bekannten Formel $y = e^{-m} \cdot \frac{m^x}{x!}$ berechnet werden können. Die Abweichung von der Poissonschen Verteilung wurde mit dem von FISCHER, THORNTON und MACKENZIE (1950) angegebenen Prüfverfahren nach $\chi^2 = \frac{1}{x} \cdot S\,(x - \bar{x})^2$ berechnet. Auch für diese Art der Berechnung wurden zunächst alle 5 langen Chromosomen in Viertelabschnitte geteilt und dann für jedes Chromosom die Zahl der Abschnitte ermittelt, in denen die gleiche Anzahl von Bruchstellen beobachtet wurde (Tabelle 5). Ebenso wurde bei der Einteilung der Chromosomen in Zehntelabschnitte (Tabelle 6) und endlich auch in 2 mm lange Abschnitte (Tabelle 7) verfahren. Durch diese Berechnung sollte ermittelt werden, ob das Auftreten von Bruchstellenkoinzidenzen zufällig ist oder nicht. Die Längeneinheit von 2 mm wurde deshalb gewählt, weil in diesem Falle ein Abschnitt etwas länger ist als die durchschnittliche Länge der Querscheibenzwischenräume auf dem Originalentwurf der Chromosomenkarte. Die Gesamtzahl der 2 mm-Abschnitte beträgt 1068 und ist somit etwas geringer

als die von KUNZE-MÜHL und MÜLLER (1958) angegebene Zahl der Querscheiben von 1460 für die 5 langen Chromosomen. Dadurch wird auch der Einwand hinfällig, daß im Ausnahmefall einer Fehlbeobachtung bei der Feststellung der Bruchstellenkoinzidenzen Bruchstellen als identisch bezeichnet werden könnten, auch wenn sie in einem Segment zusammenfielen, das etwas größer ist als die durchschnittliche Länge der Querscheibenzwischenräume.

Tabelle 5. *Frequenz natürlicher Bruchstellen in Viertelabschnitten*

Anzahl der Abschnitte	Beobachtete Anzahl der Bruchstellen pro Abschnitt												Gesamtzahl der Abschnitte	Gesamtzahl der Bruchstellen	χ^2	p
	0	1	2	2,5	4	5	5,5	6,5	7	10	11	13				
im A-Chromosom	1	2	1	0	0	0	0	0	0	0	0	0	4	4	2,00	0,70—0,50
im I-Chromosom	2	1	0	0	0	1	0	0	0	0	0	0	4	6	11,33	0,02—0,01
im U-Chromosom	0	0	0	2	1	0	0	0	1	0	0	0	4	16	3,38	0,50—0,30
im E-Chromosom	1	0	1	0	0	0	1	1	0	0	0	0	4	14	7,86	0,05—0,02
im O-Chromosom	0	0	1	0	0	0	0	0	0	1	1	1	4	36	7,78	0,10
in allen Chromosomen	4	3	3	2	1	1	1	1	1	1	1	1	20	76	32,35	0,05—0,02

Tabelle 6. *Frequenz natürlicher Bruchstellen in Zehntelabschnitten*

Anzahl der Abschnitte	Beobachtete Anzahl der Bruchstellen pro Abschnitt										Gesamtzahl der Abschnitte	Gesamtzahl der Bruchstellen	χ^2	p
	0	1	1,5	2	2,5	3	3,5	4	5	8				
im A-Chromosom	6	4	0	0	0	0	0	0	0	0	10	4	6,00	0,80—0,70
im I-Chromosom	7	1	0	1	0	1	0	0	0	0	10	6	17,33	0,05—0,02
im U-Chromosom	4	0	1	2	0	1	1	1	0	0	10	16	13,69	0,20—0,10
im E-Chromosom	3	1	0	4	2	0	0	0	0	0	10	14	7,07	0,70—0,50
im O-Chromosom	0	3	0	0	0	2	0	1	3	1	10	36	12,89	0,20—0,10
in allen Chromosomen	20	9	1	7	2	4	1	2	3	1	50	76	56,98	0,50—0,40

Aus den Tabellen 5, 6 und 7 können die folgenden Schlüsse gezogen werden. Die Berechnung für die Verteilung der natürlichen Bruchstellen in Viertelabschnitten aller langen Chromosomen ergibt ein Summen-χ-Quadrat von 32,35, dem entspricht ein p-Wert von 0,05—0,02. Diese schwache Sicherung für eine Abweichung von der Zufallsverteilung stimmt mit dem Resultat der Tabelle 3 überein, und man kann annehmen, daß die Ursache für die Abweichung nach Tabelle 5 wohl ebenfalls ausschließlich die niedere Bruchstellenfrequenz in den proximalen Vierteln ist. Die χ-Quadrate für die einzelnen Chromosomen sind sehr unterschiedlich, wobei vor allem die gute Übereinstimmung mit der Gleichverteilung in den Chromosomen A, U und O auffällt.

Tabelle 7. *Frequenz natürlicher Bruchstellen in 2 mm-Abschnitten*

Anzahl der Abschnitte	Beobachtete Anzahl der Bruchstellen pro Abschnitt						Gesamtzahl der Abschnitte	Gesamtzahl der Bruchstellen	χ^2	p
	0	1	1,5	2	2,5	3				
im A-Chromosom	176	4	0	0	0	0	180	4	196,40	0,40—0,30
im I-Chromosom	195	4	0	1	0	0	200	6	260,67	0,01—0,001
im U-Chromosom	204	8	1	2	1	0	216	16	334,86	$< 0{,}001$
im E-Chromosom	207	4	1	3	1	0	216	14	395,33	$< 0{,}001$
im O-Chromosom	227	24	0	3	3	0	2	256	36	349,86	$< 0{,}001$
in allen Chromosomen	1009	44	2	9	2	2	1068	76	1537,12	$< 0{,}001$

Die Verteilung der natürlichen Bruchstellen auf Zehntelabschnitte ist bei dieser Betrachtungsweise und Art der Berechnung (Tabelle 6) übereinstimmend mit der Gleichverteilung, wenn dieses Resultat auch nur schwach gesichert ist. Nur für das I-Chromosom kann auch bei dieser Art der Berechnung eine Abweichung von der Gleichverteilung angenommen werden. Die schwache Signifikanz für eine ungleiche Verteilung, die sich in Tabelle 4 zeigt und die durch die niedere Bruchstellenfrequenz in den proximalen Fünfteln bedingt wird, kommt wahrscheinlich bei diesem Test gar nicht mehr zum Ausdruck.

Besonders interessant aber sind die in Tabelle 7 zusammengestellten Resultate. Hier zeigt sich für alle langen Autosomen und somit auch für das Summen-χ-Quadrat eine hohe Signifikanz für ein überzufälliges Auftreten von Koinzidenzen natürlicher Bruchstellen bzw. für deren Häufung in engster Nachbarschaft.

Für die natürlichen Bruchstellen scheinen also die folgenden Ergebnisse auf Grund der oben beschriebenen statistischen Berechnungen gesichert: Erstens die niedere Bruchstellenfrequenz in den proximalen Fünfteln bzw. Vierteln des Chromosomensatzes und zweitens die überzufällige Häufigkeit von offenbar annähernd zufällig über den ganzen Chromosomensatz verteilten Bruchkoinzidenzen.

b) Verteilung der röntgeninduzierten Bruchstellen

Auch die Verteilung der röntgeninduzierten Bruchstellen wurde zunächst mittels der einfachen χ^2-Methode für die Viertel- und Zehntelabschnitte gleicher Lage aller 5 langen Chromosomen getestet. Eine gesonderte Berechnung für jedes einzelne der langen Chromosomen konnte für die Verteilung der röntgeninduzierten Bruchstellen auf Zehntelabschnitte bei diesem Testverfahren wegen der geringen Erwartungszahlen nicht durchgeführt werden.

Die Ergebnisse der Tabellen 8 und 9 zeigen, daß sowohl die Verteilung auf Viertel- als auch auf Zehntelabschnitte gut mit einer Gleichverteilung übereinstimmt, d. h. daß in bestimmten Abschnitten, die in jedem der 5 langen Chromosomen gleiche Lage besitzen, keine zu hohe oder zu niedere Bruchstellenfrequenz auftritt.

Tabelle 8. *Frequenz röntgeninduzierter Bruchstellen in Viertelabschnitten von gleicher Lage*

Chromosom	Beobachtete Anzahl der Bruchstellen im				Gesamtzahl der Bruchstellen	χ^2	p
	1. Viertel prox.	2. Viertel	3. Viertel	4. Viertel			
A	11	5	7	6	29	2,87	0,50—0,30
I	12	8	21	14	55	6,44	0,10—0,05
U	11	11	15	10	47	1,26	0,80—0,70
E	9	8	9	12	38	0,96	0,90—0,80
O	10	23	13	19	65	6,32	0,10—0,05
A—O	53	55	65	61	234	1,56	0,70—0,50

Tabelle 9. *Frequenz röntgeninduzierter Bruchstellen in Zehntelabschnitten gleicher Lage*

Chromosom	Beobachtete Anzahl der Bruchstellen im										Gesamtzahl der Bruchstellen	χ^2	p
	1. Zehntel prox.	2. Zehntel	3.	4.	5.	6.	7.	8.	9.	10.			
A	5	4	5	1	2	0	4	3	3	2	29		
I	2	6	5	3	4	13	5	6	11	0	55		
U	3	6	6	3	3	4	8	6	2	5	47		
E	5	4	0	5	3	7	2	4	7	1	38		
O	5	2	9	6	11	5	2	15	5	5	65		
A—O	20	22	25	18	24	29	21	34	28	13	234	13,86	0,20—0,10

Überraschend sind die Resultate, wenn man die Verteilung in Viertel-, Zehntel- und 2 mm-Abschnitten gegenüber der Poissonschen Verteilung mittels der oben bereits beschriebenen Methode prüft.

Wie aus Tabelle 10 ersichtlich ist, ergeben sowohl die χ-Quadrate für die einzelnen Chromosomen sowie das Summen-χ-Quadrat nicht signifikante p-Werte. Das bedeutet, daß in den langen Chromosomen keine Viertelabschnitte vorhanden sind, in denen eine extrem hohe oder besonders niedere Anzahl von Bruchstellen vorliegt.

Tabelle 11 zeigt, daß die Verteilung der Bruchstellen auf Zehntelabschnitte in den Chromosomen I und O nicht dem Zufall entspricht, während eine Zufallsverteilung in den Chromosomen A, E und U angenommen werden kann. Das Summen-χ-Quadrat ergibt einen signifikanten p-Wert für die Abweichung von der Zufallsverteilung.

Die Berechnung für die Verteilung röntgeninduzierter Bruchstellen in 2 mm langen Abschnitten zeigt eine Übereinstimmung mit der Gleichverteilung nur für die Chromosomen A und E, übereinstimmend mit den Resultaten in den Tabellen 10 und 11. In den übrigen Chromosomen treten nicht zufällige Häufungen von Bruchstellen auf, und auch

Tabelle 10. *Frequenz röntgeninduzierter Bruchstellen in Viertelabschnitten*

Anzahl der Abschnitte	Beobachtete Anzahl der Bruchstellen pro Abschnitt															Gesamtzahl der Abschnitte	Gesamtzahl der Bruchstellen	χ^2	p
	0	5	6	7	8	9	10	11	12	13	14	15	19	21	23				
im A-Chromosom	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	4	29	2,86	0,50—0,30
im I-Chromosom	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	4	55	6,45	0,10—0,05
im U-Chromosom	0	0	0	0	0	0	1	2	0	0	0	1	0	0	0	4	47	1,26	0,80—0,70
im E-Chromosom	0	0	0	0	1	2	0	0	1	0	0	0	0	0	0	4	38	0,95	0,90—0,80
im O-Chromosom	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	4	65	6,32	0,10—0,05
in allen Chromosomen		1	1	1	2	2	2	3	2	1	1	1	1	1	1	20	234	17,85	0,70—0,50

Tabelle 11. *Frequenz röntgeninduzierter Bruchstellen in Zehntelabschnitten*

Anzahl der Abschnitte	Beobachtete Anzahl der Bruchstellen pro Abschnitt													Gesamtzahl der Abschnitte	Gesamtzahl der Bruchstellen	χ^2	p
	0	1	2	3	4	5	6	7	8	9	11	13	15				
im A-Chromosom	1	1	2	2	2	2	0	0	0	0	0	0	0	10	29	8,59	0,50—0,30
im I-Chromosom	1	0	1	1	1	2	2	0	0	0	1	1	0	10	55	25,18	0,01—0,001
im U-Chromosom	0	0	1	2	2	1	3	0	1	0	0	0	0	10	47	5,98	0,80—0,70
im E-Chromosom	1	1	1	1	2	2	0	2	0	0	0	0	0	10	38	13,05	0,20—0,10
im O-Chromosom	0	0	2	0	0	4	1	0	0	1	1	0	1	10	65	22,84	0,01—0,001
in allen Chromosomen	3	2	7	6	7	11	6	2	1	1	2	1	1	50	234	75,64	0,02—0,01

das Summen-χ-Quadrat ergibt einen hoch signifikanten p-Wert (Tabelle 12). Es ist wahrscheinlich, daß die Häufigkeit von Bruchstellenkoinzidenzen hauptverantwortlich für die Abweichung von der Gleichverteilung in den Zehntel- und 2 mm-Abschnitten gemacht werden kann. Auf jeden Fall kann ausgesagt werden, daß es im Chromosomensatz von *Drosophila subobscura* kleinere und sehr kleine Abschnitte gibt, in denen Häufungen von röntgeninduzierten Bruchstellen vorkommen, und daß diese Abweichung von einer Zufallsverteilung bei der Verwendung kleinster Abschnitte als Berechnungsgrundlage statistisch deutlich zum Ausdruck kommt. Hingegen fehlt in der Verteilung der

Tabelle 12. *Frequenz röntgeninduzierter Bruchstellen in 2 mm-Abschnitten*

Anzahl der Abschnitte	Beobachtete Anzahl der Bruchstellen pro Abschnitt					Gesamtzahl der Bruchstellen	Gesamtzahl der Abschnitte	χ^2	p
	0	1	2	3	4				
im A-Chromosom	154	23	3	0	0	29	180	188,42	0,60—0,50
im I-Chromosom	160	29	8	2	1	55	200	290,45	< 0,001
im U-Chromosom	177	33	4	2	0	47	216	260,25	0,05—0,02
im E-Chromosom	180	34	2	0	0	38	216	200,67	0,60—0,50
im O-Chromosom	208	34	11	3	0	65	256	348,36	< 0,001
in allen Chromosomen	879	153	28	7	1	234	1068	1288,15	< 0,001

strahleninduzierten Bruchstellen die bei den natürlichen Bruchstellen auffallende relative Frequenzsenkung in den proximalen Chromosomenabschnitten.

Als ein sehr wesentliches Ergebnis erscheint die Feststellung, daß sowohl bei den Bruchstellen natürlicher Inversionen als auch bei den strahleninduzierten Bruchstellen die überzufällige Häufigkeit von Bruchstellenkoinzidenzen offenbar die Ursache für die Abweichung von der Zufallsverteilung bei Prüfung in kleinen Chromosomenabschnitten ist. Man könnte vermuten, daß dieses interessante Phänomen bei den natürlichen und bei den röntgeninduzierten Bruchstellen auf einer gemeinsamen Grundlage beruht, wenn der Nachweis einer überzufälligen Häufung von Koinzidenzstellen zwischen beiden Bruchgattungen erbracht werden könnte. Wäre innerhalb der beiden Bruchgattungen eine Gleichverteilung auf 2 mm-Abschnitte vorhanden, so könnte die Prüfung auf ein zufälliges Auftreten von Koinzidenzen zwischen natürlichen und strahleninduzierten Bruchstellen mit dem oben beschriebenen Testverfahren erfolgen. Da dies nicht der Fall ist, kann eine Berechnung dieser Art nicht durchgeführt werden. Weitere Schwierigkeiten für eine statistische Prüfung dieser Frage ergeben sich daraus, daß die natürlichen Bruchstellenkoinzidenzen nicht in allen Fällen ohne weiteres mit den Koinzidenzen röntgeninduzierter Bruchstellen vergleichbar sind. Außerdem liegt die Zahl der natürlichen Bruchstellen fest, während die Zahl der strahleninduzierten Bruchstellen beliebig erhöht werden kann. Diese Zahl ist in der vorliegenden Untersuchung mehr als dreimal so hoch als die der natürlichen Bruchstellen. Alle diese Umstände komplizieren die Aufgabe so ungemein, daß es nicht möglich war, ein geeignetes statistisches Prüfverfahren zur Lösung dieses Problems zu finden. Auf jeden Fall aber scheint die Zahl der Koinzidenzen zwischen natürlichen und röntgeninduzierten Bruchstellen höher zu sein als bei anderen *Drosophila*-Arten (Helfer 1941, Koller und Ahmed 1942). Wie aus dem Schema ersichtlich ist, fallen bei *Drosophila subobscura*

je zwei Bruchenden an einer natürlichen Bruchstelle 9mal mit je einem, 4mal mit je zwei und 1mal mit drei röntgeninduzierten Bruchstellen zusammen. Weiter koinzidieren je einmal 3 Bruchenden natürlicher Inversionen mit einer, 5 Bruchenden mit einer und 6 Bruchenden mit zwei strahleninduzierten Bruchstellen. Eine exakte Aussage darüber, ob das Auftreten dieser Koinzidenzen noch zufällig ist oder nicht, kann allerdings ohne statistische Berechnungen nicht gemacht werden.

Diskussion der Ergebnisse

Der Aussagewert dieser Untersuchungen über die Verteilung strahleninduzierter Bruchstellen ist zunächst durch die Wahl des Objektes und der Methode eingegrenzt. Die Primärbrüche entstehen in den reifen Spermien, in deren Kopf die Chromosomen in einem spezialisierten Zustand und in einer bestimmten Anordnung bei zufälliger Verteilung vorliegen dürften. Vieles spricht dafür, daß die Verheilung von Primärbrüchen und damit auch die dislozierte Verheilung von Bruchenden während der Auflockerung des Spermienkerns zum männlichen Vorkern stattfindet und daß dabei der Rekombinationsbereich für die Entstehung von Dislokationen einen bestimmten Umfang hat. Bauer (1939) hat schon auf Grund seiner Untersuchungen an *Drosophila melanogaster* diese Verhältnisse erörtert, und er erklärt mit der Beschränkung des Rekombinationsbereiches die Erscheinung, daß mit dem auch hier für *Drosophila subobscura* bestätigten Verhältnis von ungefähr 2:1 zwischen Translokationen und Inversionen die relative Häufigkeit der Inversionen gegenüber der Zufallserwartung zu hoch ist. Aus der Zahl und der Verteilung der in den Dislokationen erfaßten Bruchstellen kann man nicht ohne weiteres auf die Zahl und die Verteilung der Primärbrüche schließen, da wir nicht wissen, inwieweit die Aussichten für eine dislozierte Verheilung für alle primären Bruchenden oder in allen Bereichen der verschiedenen Chromosomen die gleichen sind. Weiter ist zu bedenken, daß mit der hier verwendeten Methode nur alle jene Dislokationen erfaßt werden konnten, die das Leben der Larve erfolgreich bis zur Verpuppungsreife zuließen, während Dislokationen, von denen etwa im heterozygoten Zustand eine letale oder subvitale Wirkung ausging, sich der Feststellung entzogen. Das hier beobachtete Muster der Verteilung von strahleninduzierten Bruchstellen kann daher nicht ohne weiteres als ein Abbild der Verteilung der Primärbrüche aufgefaßt werden und gibt vielleicht auch kein unverändertes Bild der Verteilung der Rekombinationsstellen wieder. Unmittelbar lassen sich die vorliegenden Ergebnisse daher nur mit solchen vergleichen, die mit verwandten Objekten und mit der gleichen Methode gewonnen worden sind.

Die Feststellung, daß die strahleninduzierten Bruchstellen bei *Drosophila subobscura* proportional der Länge der voll ausgebildeten Speichel-

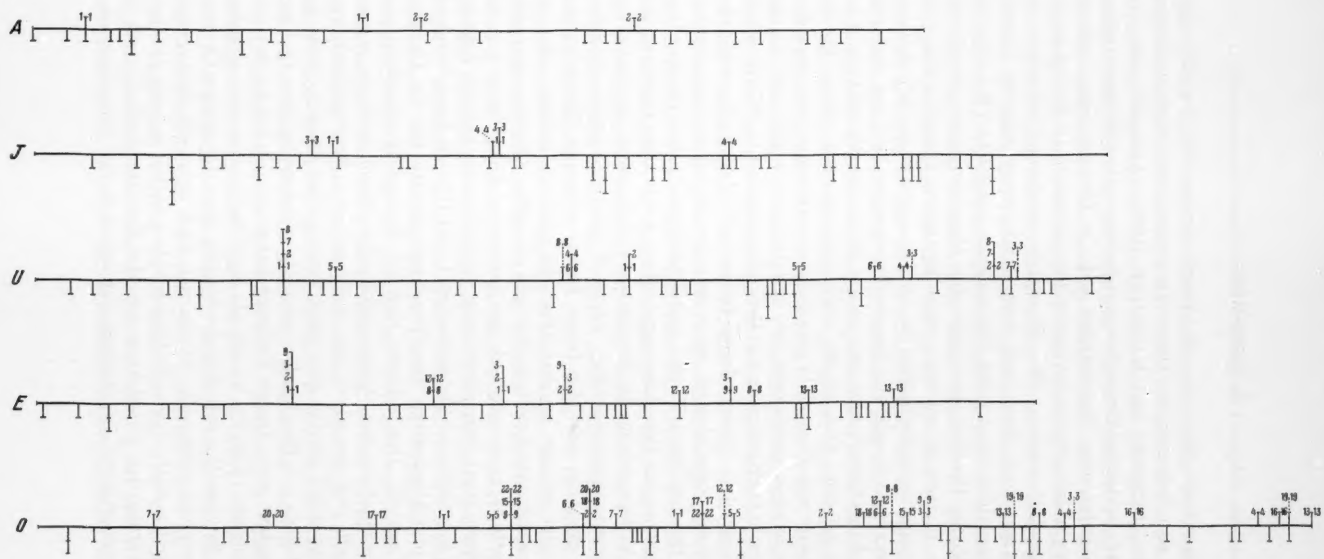

Abb. 1. Schematische Darstellung der Chromosomen *A, J, U, E* und *O* von *Drosophila subobscura* in richtiger Längenproportion. Die Lage der röntgeninduzierten Bruchstellen ist durch senkrechte Markierung nach unten eingezeichnet, wobei im Falle von äquilokalen Bruchstellen die senkrechten Marken entsprechend verlängert wurden. Die Bruchenden natürlicher Inversionen sind als senkrechte Linien nach oben eingetragen und mit den auf der Chromosomenkarte (KUNZE-MÜHL und MÜLLER 1958) verwendeten Nummern der Inversionen versehen

drüsenchromosomen über die vier langen Autosomen verteilt sind, stimmt mit den Befunden bei *Drosophila melanogaster* überein (BAUER 1939, BAUER, DEMEREC und KAUFMANN 1938). Auch KOLLER und AHMED (1942) fanden bei *Drosophila pseudoobscura* eine Gleichverteilung von strahleninduzierten Bruchstellen auf die Chromosomen, solange nicht kleinere Abschnitte als die ganzen Chromosomen als Berechnungsgrundlage gewählt wurden. Bei *Drosophila subobscura* entspricht die Verteilung sogar auch dann noch dem Zufall, wenn man die Viertelabschnitte innerhalb eines jeden Chromosoms vergleicht. Bei allen bis jetzt angeführten Berechnungen wäre eine Abweichung von der Gleichverteilung z. B. dann zu erwarten, wenn bestimmte Chromosomen oder größere Chromosomenabschnitte, die in den natürlichen Populationen eine besonders hohe Anzahl von Spontanbruchstellen besitzen, auch eine höhere Bruchanfälligkeit oder eine höhere Rekombinationsfähigkeit bei Bestrahlung zeigten. Dies wurde schon von HELFER (1941) für das III. Chromosom von *Drosophila pseudoobscura* widerlegt, das besonders reich an natürlichen Inversionen ist, und das gleiche gilt auch für das hochpolymorphe O-Chromosom von *Drosophila subobscura*. Weiter wurde in der vorliegenden Untersuchung festgestellt, daß auch dann eine Gleichverteilung vorhanden ist, wenn man die Verteilung von strahleninduzierten Bruchstellen in Viertel- und Zehntelabschnitten gleicher Lage in den Autosomen als Berechnungsgrundlage wählt. Das bedeutet, daß keinerlei Anhaltspunkte für die Annahme vorhanden sind, daß bestimmte größere Chromosomenabschnitte, wie z. B. die chromozentrumnahen Zonen oder etwa die nahe den freien Enden gelegenen Chromosomenteile, mehr oder weniger bruchanfällig sind bzw. eine größere oder geringere Rekombinationsbereitschaft zeigen als andere Chromosomenteile.

Eine deutliche und statistisch gut gesicherte Abweichung von der Gleichverteilung ergab sich jedoch beim Vergleich zwischen den Zehntelabschnitten innerhalb der Chromosomen I und O sowie beim Vergleich zwischen 2 mm-Abschnitten der Chromosomenkarte für die Chromosomen I, U und O. Das bedeutet, daß bei *Drosophila subobscura* eine überzufällige Häufung von strahleninduzierten Bruchstellen in engster Nachbarschaft und vor allem das Auftreten von Bruchstellenkoinzidenzen festzustellen ist und daß diese Bereiche der Häufung bzw. des Zusammenfallens von Bruchstellen ungefähr gleichmäßig über die Chromosomen verteilt sind. Diesen Eindruck hat man auch bei der Betrachtung des Schemas (Abb. 1), wo die als äquilokal bestimmten strahleninduzierten Bruchstellen ziemlich gleichmäßig über den ganzen Chromosomensatz verteilt erscheinen. Wenn man auch gegen die Bestimmung der Bruchstellen methodische Bedenken haben wollte, so zeigt die statistische Sicherung der Abweichung von der Zufallsverteilung auch bei der Wahl von größeren Abschnitten zumindest in den Chromosomen I

und O, daß eine eventuelle Fehlbestimmung einer Koinzidenz für das Ergebnis keine Rolle spielen kann. Das Auftreten von solchen strahleninduzierten äquilokalen Bruchstellen wurde bei anderen *Drosophila*-Arten sowie auch bei *Chironomiden* niemals besonders hervorgehoben. Die Autoren solcher Berichte sprechen meist von einer überzufälligen Häufung von strahleninduzierten Bruchstellen in bestimmten Zonen oder Unterzonen der entsprechenden Chromosomenkarten. HELFER (1941) z. B. fand unter 73 strahleninduzierten Bruchstellen des III. Chromosoms von *Drosophila pseudoobscura* keine einzige Koinzidenz. KOLLER und AHMED (1942) beobachteten unter weiteren 79 Bruchstellen des gleichen Chromosoms nur eine, die in der gleichen Unterzone lag, wie eine von HELFER festgestellte Bruchstelle und die als vermutlich identisch mit dieser bezeichnet wird. Es wurde also bei *Drosophila pseudoobscura* unter 152 durch Röntgenstrahlen erzeugten Bruchstellen nur eine einzige vermutliche Koinzidenz gefunden. Auch KAUFMANN (1939) und PROKOFYEVA-BELGOVSKAYA und KVOSTOVA (1939) haben bei sorgfältigen Untersuchungen über die Verteilung strahleninduzierter Bruchstellen über das X-Chromosom von *Drosophila melanogaster* wohl eine gesicherte Abweichung von der Zufallsverteilung dann gefunden, wenn die Chromosomenlänge in kleine Unterabschnitte eingeteilt wird, sprechen jedoch niemals von echten Koinzidenzen, sondern von einer Häufung von Bruchstellen in bestimmten engen Bereichen. Sie nehmen an, daß dieses auffallende Phänomen auf dem Vorhandensein von interkalarem Heterochromatin beruhe und stützen diese Vermutung auf die Tatsache, daß an solchen Stellen häufig Verklebungen der Chromosomen untereinander vorkommen. Derartige Erscheinungen konnten aber bei *Drosophila subobscura* nicht beobachtet werden. Man könnte allerdings annehmen, daß bei *Drosophila subobscura* jene Zonen, in denen überzufällige Häufungen von strahleninduzierten Bruchstellen auftreten, viel längeren heterochromatischen Abschnitten in den Mitosechromosomen entsprechen, wie das von BAUER (1939) für *Drosophila melanogaster* festgestellt wurde, wo die proximalen heterochromatischen Zonen der Chromosomen eine ihrer Längsausbildung in den Mitosechromosomen entsprechende Bruchhäufigkeit aufweisen. Allerdings würde diese Annahme nicht das Auftreten von strengen Bruchkoinzidenzen bei *Drosophila subobscura* erklären. Auch bei *Chironomiden* wurden Häufungen von strahleninduzierten Bruchstellen in bestimmten Unterzonen der Chromosomen festgestellt. So berichtet z. B. KEYL (1958) von diesem Phänomen bei *Chironomus thummi*. Allerdings kann diese Untersuchung nicht unmittelbar mit den erwähnten Untersuchungen an *Drosophila*-Arten verglichen werden, da es sich hier um eine Bestrahlung von Embryonalstadien und nicht um eine Bestrahlung der Spermien handelt. Immerhin treten auch hier an bestimmten augenscheinlich nicht hetero-

chromatischen Stellen Häufungen von Rekombinationsbrüchen auf, wobei auch hier niemals ausdrücklich von Bruchstellenkoinzidenzen gesprochen wird.

Für das auffallende Phänomen des Auftretens von Bruchstellenkoinzidenzen bei *Drosophila subobscura* gäbe die folgende Deutung eine sehr gute Erklärung. Man könnte annehmen, daß zwar die Verteilung der Primärbrüche bzw. der Rekombinationsstellen zunächst eine rein zufällige ist, daß aber von einigen Dislokationen in gewissen Zonen der Chromosomen im heterozygoten Zustand letale oder subvitale Wirkungen im Sinne eines Positionseffektes ausgehen und die Träger solcher Dislokationen daher nicht zur Präparation gelangen. Von den als Koinzidenzen erscheinenden Bruchstellen könnte ein günstiger Positionseffekt ausgeübt werden, wodurch die Träger solcher Dislokationen bevorzugt zur Verarbeitung kommen. Für diese Annahme würde vor allem die auffallende und sonst nicht deutbare Feststellung sprechen, daß bei der gleichen Strahlungsdosis der Prozentsatz der Diskolationen zeigenden F_1-Larven bei *Drosophila subobscura* viel kleiner ist als bei den obengenannten *Drosophila*-Arten, obzwar die durchschnittliche Anzahl der Brüche pro betroffenes Spermium die gleiche ist. Da zur Erklärung der Häufigkeit von äquilokalen Bruchstellen im natürlichen Inversionspolymorphismus von *Drosophila subobscura* die gleiche Vorstellung bereits entwickelt worden ist, wäre es eine besondere Stütze für diese Annahme, wenn eine überzufällige Häufung von Koinzidenzen zwischen den strahleninduzierten Bruchstellen und den Bruchenden natürlicher Inversionen nachgewiesen werden könnte. Wenn man auch bei Betrachtung des Schemas (Abb. 1) diesen Eindruck hat, so konnte mangels einer geeigneten statistischen Methode kein strikter Beweis dafür erbracht werden. Als ein weiterer Hinweis auf die Brauchbarkeit der Positionseffekthypothese kann aber der von Sperlich (1959) mitgeteilte Fall gewertet werden, in dem ein durch Bestrahlung erzeugter Strukturtyp des U-Chromosoms von *Drosophila subobscura* sich sofort als selektiv erfolgreich erwies. Die durch Bestrahlung neu entstandene Inversion dieses Strukturtyps hatte ein Bruchende mit mehreren natürlichen Inversionen gemeinsam.

Die Frage der Verteilung der Bruchenden natürlicher Inversionen, die in dieser Untersuchung ebenfalls einer statistischen Bearbeitung unterworfen wurde, hat ganz andere Voraussetzungen als die bisher besprochenen Fragen der strahleninduzierten Brüche. Während erfahrungsgemäß fast alle strahleninduzierten Dislokationen im Laufe von einer oder zwei Generationen aus der Nachkommenschaft der bestrahlten Tiere restlos verschwinden, sichtlich also schädlich oder zumindest nicht vorteilhaft sind, handelt es sich bei den natürlichen Inversionen um Bildungen, die den sie tragenden Strukturtypen einen Selektionsvorteil

im strukturheterozygoten Zustand sichern und auf diese Weise zum stabilen Bestandteil der Population geworden sind. Von der großen Mannigfaltigkeit der Inversionen, die im Laufe der Phylogenie zufällig entstanden sein mögen, liegt hier nur eine sehr geringe, durch die Selektion getroffene Auswahl vor. Man kann daher keinesfalls erwarten, daß die Verteilung ihrer Bruchenden über den Chromosomensatz irgendeinen Aufschluß über die Verteilung von primären Brüchen oder Rekombinationen gibt.

Die ungleiche Verteilung der Inversionen auf die verschiedenen Chromosomen, die bei *Drosophila subobscura* sehr deutlich in Erscheinung tritt, ist bei vielen chromosomal polymorphen *Drosophila*-Arten festzustellen (s. die Zusammenstellung bei MAINX 1956) und auch bei einigen anderen *Dipteren*, z. B. *Tendipes decorus* (ROTHFELS und FAIRLIE 1957). In extremen Fällen sind fast alle Inversionen auf einem Chromosom versammelt wie bei *Drosophila nebulosa* und *Drosophila prosaltans*. Bei anderen Arten sind allerdings die Inversionen ungefähr gleichmäßig über die euchromatischen Bereiche aller Chromosomen verteilt wie bei *Drosophila algonquin*, *Drosophila paulistorum* und *Drosophila willistoni*. Innerhalb der *obscura*-Gruppe der Gattung *Drosophila* können wir eine gleichmäßige Verteilung der Inversionen bei *Drosophila algonquin* und *Drosophila azteka* deren Häufung auf dem III. Chromosom von *Drosophila pseudoobscura* und *Drosophila persimilis*, auf dem C-Chromosom von *Drosophila athabasca* und dem O-Chromosom bei *Drosophila subobscura* gegenüberstellen. Diese Chromosomen dürften auch nach ihrem Genbestand phylogenetisch homologe Chromosomen sein. Die Häufung der Inversionen auf diesen Elementen bei verschiedenen Arten dieser Gruppe ist also wohl kein Zufall. Bei *Drosophila subobscura* scheinen keine intrachromosomalen Beziehungen die Selektionswerte der Heterozygoten zu beeinflussen, wie aus den Feststellungen in künstlichen Populationen (SPERLICH 1958), aber auch aus der Analyse natürlicher Populationen (KUNZE-MÜHL, MÜLLER und SPERLICH 1958) hervorgeht. Bis auf allgemeine Vermutungen über wesentliche Unterschiede zwischen den verschiedenen Chromosomen in ihrer strukturell-genischen Dynamik läßt sich wohl für die Erscheinung der ungleichen Verteilung der Inversionen auf die Chromosomen heute noch keine Erklärung geben.

Vergleicht man bei *Drosophila subobscura* die Verteilung der natürlichen Bruchenden zwischen den Viertel- und Zehntelabschnitten innerhalb eines jeden Chromosoms, so ergibt sich dann eine Gleichverteilung, wenn man die proximalen, d. h. centromernahen Viertel bzw. Fünftel bei der Berechnung vernachlässigt. Diese Chromosomenteile sind bei *Drosophila subobscura* ziemlich arm an Bruchstellen natürlicher Inversionen. Bei anderen Arten der *obscura*-Gruppe, wie im III. Chromosom von *Drosophila pesudoobscura* und *Drosophila athabasca*, ist die Häufung

der Bruchenden im distalen Chromosomenabschnitt noch viel deutlicher ausgeprägt, ebenso auch bei *Drosophila nebulosa* und im III. Chromosom von *Drosophila willistoni*, während bei anderen Chromosomen der letztgenannten Art eine gleichmäßige Verteilung herrscht. Ob diese Erscheinungen auf chromosomal-mechanischen Ursachen oder auf genischen Verhältnissen beruhen, kann wohl nicht entschieden werden. Jedenfalls müssen wir auch hier stets daran denken, daß das Verteilungsmuster der Inversionen im natürlichen Polymorphismus das Ergebnis des Einbaues selektiv erfolgreicher Strukturtypen in die Population ist und uns daher niemals ein unverändertes Bild von rein zufällig entstandenen Dislokationen oder von rein mechanisch bedingten Verteilungen bieten kann.

Besonders auffallend ist der Beweis der signifikanten Abweichung von der Zufallsverteilung der Bruchenden natürlicher Inversionen, der sich im Vergleich von Zehntelabschnitten für das I-Chromosom, beim Vergleich von 2 mm-Abschnitten innerhalb der Chromosomen aber für alle Autosomen von *Drosophila subobscura* ergibt. Es ist klar, daß daran wieder die relativ hohe Zahl von äquilokalen Bruchstellen schuld ist. Diese Erscheinung scheint in den natürlichen Populationen keiner anderen *Drosophila*-Art so extrem ausgeprägt zu sein wie bei *Drosophila subobscura*. Wenn auch bei einigen *Drosophila*-Arten und manchen *Chironomiden* auffallende Häufungen natürlicher Bruchstellen in enger Nachbarschaft angegeben werden, so sind echte Koinzidenzen im Bereich zwischen 2 Querscheiben nur relativ selten gefunden worden. Dobzhansky und Epling (1944) geben an, daß bei *Drosophila pseudoobscura* unter 21 Inversionen je eine Bruchstelle dreier Inversionen in dem sehr kurzen Segment 70B—71A liegt. Novitsky (1946) macht keine konkreten Angaben über Bruchstellenkoinzidenzen bei *Drosophila athabasca*, jedoch scheinen auch hier einige äquilokale Bruchstellen vorzuliegen, da in Fig. 11a nur 27 Bruchstellen für 32 Brüche eingezeichnet sind. Weiter berichtet Ward (1952), daß unter 16 Inversionen im II. Chromosom von *Drosophila melanica* strenge Koinzidenzen für die proximalen Brüche der Inversionen a und m, für den distalen der Inversion e und den proximalen der Inversion f sowie für den distalen von f und den proximalen von g gefunden werden konnten. Auch wurden bei dieser Art mehrere Bruchstellen beschrieben, die in engster Nachbarschaft liegen. Eine auffallende Häufung natürlicher Bruchstellen in bestimmten sehr kleinen Bruchsegmenten wurde auch bei *Tendipes decorus* von Rothfels und Fairlie (1957) beschrieben. Schon das Auftreten der oben beschriebenen Bruchstellenkoinzidenzen kann nicht rein zufallsmäßig bedingt sein, und dies gilt in erhöhtem Maß für *Drosophila subobscura*, wo unter 152 Bruchenden Koinzidenzen von 3 Bruchenden 2mal, von 4 Bruchenden 9mal, von 5 Bruchenden 2mal und von 6 Bruchenden ebenfalls 2mal vorkommen.

Zur Deutung dieser Erscheinung wurde öfters die Hypothese von NOVITSKY (1946) herangezogen, die für die Häufung und charakteristische Verteilung der Bruchstellen in bestimmten Bereichen des III. Chromosoms von *Drosophila athabasca* und *Drosophila pseudoobscura* eine ansprechende Erklärung gibt und die auch von ROTHFELS und FAIRLIE (l.c.) für ihre Befunde an *Tendipes* angewendet wird. Daß diese Hypothese im Bereich des chromosomalen Polymorphismus der *Dipteren* nicht allgemein anwendbar ist, hat MAINX (1956) schon diskutiert. Der wesentliche Einwand ist wohl der, daß die Hypothese mit rein chromosomal-mechanischen Vorstellungen arbeitet, um die Entstehung der Inversionen im „Tandem" zu erklären, damit aber keine Rechtfertigung dafür liefert, warum die so entstandenen Strukturtypen selektiv erfolgreich sein sollten und daher zum Einbau in die Population gelangten. Aus diesen und anderen Gründen haben KUNZE-MÜHL und SPERLICH (1955) diese Hypothese als nicht ganz befriedigend für die Deutung der Verhältnisse bei *Drosophila subobscura* angesehen und geben einer Vorstellung den Vorzug, die für Brüche an ganz bestimmten Punkten der Chromosomen einen günstigen Positionseffekt im Sinne einer Heterosiswirkung annimmt. Die experimentellen und statistischen Feststellungen in dieser Untersuchung können als weiterer Beitrag zu dieser Hypothese gewertet werden.

Zusammenfassung

1. Zehn Tage alte Männchen des strukturell homozygoten Stammes „Küsnacht" von *Drosophila subobscura* wurden mit 5000 r röntgenbestrahlt und mit unbegatteten Weibchen gepaart. Die Speicheldrüsen von 646 Larven der F_1-Generation wurden präpariert. Bei 92 Larven wurden insgesamt 74 Translokationen und 30 Inversionen festgestellt. Diese 104 Dislokationen gehen auf 234 Bruchstellen zurück, deren Lage nach der von KUNZE-MÜHL und MÜLLER (1958) veröffentlichten Chromosomenkarte genau lokalisiert wurde.

2. Der Prozentsatz der von Dislokationen betroffenen Spermien beträgt $14{,}24 \pm 1{,}37\,\%$ und ist somit wesentlich niederer als bei allen bisher untersuchten *Drosophila*-Arten bei gleicher Strahlungsdosis. Die durchschnittliche Bruchstellenanzahl ist dagegen mit 2,54 fast ebenso hoch wie bei *Drosophila melanogaster und Drosophila pseudoobscura*.

3. Die Verteilung der 234 strahleninduzierten Bruchstellen und der 152 Bruchenden natürlicher Inversionen auf die Chromosomen und innerhalb der Chromosomen wurde mit geeigneten statistischen Methoden geprüft.

4. Bei den strahleninduzierten Bruchstellen zeigt sich eine Zufallsverteilung auf die Chromosomen und auch innerhalb der Chromosomen

beim Vergleich zwischen größeren Abschnitten. Beim Vergleich zwischen kurzen Abschnitten zeigen sich jedoch bei 3 Autosomen signifikante Abweichungen von der Gleichverteilung. Diese gehen vor allem auf das gehäufte Auftreten von äquilokalen Brüchen an bestimmten Stellen zurück.

5. Die Verteilung der natürlichen Inversionen auf die verschiedenen Chromosomen ist sehr ungleich. Außerdem sind die proximalen Abschnitte aller Autosomen ärmer an Bruchstellen als die übrigen Abschnitte. Beim Vergleich zwischen kurzen Abschnitten der Autosomen zeigt sich wieder eine signifikante Abweichung von der Gleichverteilung, die hier noch deutlicher durch die Häufung von Koinzidenzen der natürlichen Bruchenden bedingt erscheint. Eine überzufällige Koinzidenz von strahleninduzierten und natürlichen Bruchstellen wird vermutet, konnte aber nicht bewiesen werden.

6. Die Deutung dieser Ergebnisse im Vergleich mit anderen Untersuchungen wird diskutiert.

Literatur

Bauer, H.: Röntgenauslösung von Chromosomenmutationen bei *Drosophila melanogaster*. I. Chromosoma (Berl.) **1**, 343—390 (1939).

—, M. Demerec and B. P. Kaufmann: X-ray-induced chromosomal alterations in *Drosophila melanogaster*. Genetics **23**, 610—630 (1938).

Catcheside, D. G.: The effect of X-ray dosage upon frequency of induced structural changes in the chromosomes of *Drosophila melanogaster*. J. Genet. **36**, 307—320 (1938).

Dobzhansky, Th., and C. Epling: Contributions to the genetics taxonomy and ecology of *Drosophila pseudoobscura* and its relatives. Publ. Carneg. Instit. **554**, 183 (1944).

—, and A. H. Sturtevant: Inversions in the chromosomes of *Drosophila pseudoobscura*. Genetics **23**, 28—64 (1938).

Fiala, Y., u. J. Neubert: Die Erzeugung von Chromosomendislokationen durch Röntgenstrahlen in der Keimbahn und in den Geschlechtszellen verschiedenen Alters bei *Drosophila hydei Sturt*. Chromosoma (Berl.) **4**, 577—584 (1952).

Fisher, R. A., H. G. Thornton and W. A. Mackenzie: The accuracy of the plating method of estimating the density of bacterial populations. In R. A. Fisher, Contributions to mathematical statistics, p. 325—359. London 1950.

Helfer, R. G.: A comparison of X-ray-induced and naturally occurring chromosomal variations in *Drosophila pseudoobscura*. Genetics **26**, 1—22 (1941).

Kaufmann, B. P.: Distribution of induced breaks along the X-chromosome of *Drosophila melanogaster*. Proc. VII. Genet. Congr. p. 172—173 (1939). — Organization of the chromosome. I. Break distribution and chromosome recombination in *Drosophila melanogaster*. J. exp. Zool. **102**, 293—320 (1946).

—, H. Gay and H. Rothberg jr.: The influence of near infrared radiation on the production by nitrogen mustard of chromosome rearrangements in *Drosophila*. J. exp. Zool. **111**, 414—435 (1949).

Keyl, H. G.: Untersuchungen am Karyotypus von *Chironomus thummi*. II. Mitteilung: Strukturänderungen an den Speicheldrüsenchromosomen nach Röntgenbestrahlung von Embryonen und Larven. Chromosoma (Berl.) **9**, 441—483 (1958).

KOLLER, P. C., and I. A. R. S. AHMED: X-ray-induced structural changes in the chromosomes of *Drosophila pseudoobscura*. J. Genet. **44**, 53—72 (1942).

KUNZE-MÜHL, E., u. E. MÜLLER: Weitere Untersuchungen über die chromosomale Struktur und die natürlichen Strukturtypen von *Drosophila subobscura Coll.* Chromosoma (Berl.) **9**, 559—570 (1958).

— — u. D. SPERLICH: Qualitative, quantitative und jahreszeitliche Untersuchungen über den chromosomalen Polymorphismus natürlicher Populationen von *Drosophila subobscura Coll.* Z. indukt. Abstamm.- u. Vererb.-Lehre **89**, 636—646 (1958).

— u. D. SPERLICH: Inversionen und chromosomale Strukturtypen bei *Drosophila subobscura Coll.* Z. indukt. Abstamm.- u. Vererb.-Lehre **87**, 65—84 (1955).

MAINX, F.: Der chromosomale Strukturpolymorphismus natürlicher Populationen als Problem der Genetik und der Evolution. Novant'anni delle leggi Mendeliane et L. GEDDA. Roma 1956.

—, TH. KOSKE u. E. SMITAL: Untersuchungen über die chromosomale Struktur europäischer Vertreter der *obscura*-Gruppe. Z. indukt. Abstamm.- u. Vererb.-Lehre **87**, 65—84 (1955).

MATHER, K.: Statistische Analysen in der Biologie. London 1946.

NOVITSKY, E.: Chromosome variation in *Drosophila athabasca*. Genetics **31**, 508—524 (1946).

PROKOFYEVA-BELGOVSKAYA, A. A., and V. V. KHVOSTOVA: Distribution of breaks in the X-chromosome of *Drosophila melanogaster*. C. R. Acad. Sci. URSS. **23**, 270—272 (1939).

ROTHFELS, K. H., and T. W. FAIRLIE: The non-random distribution of inversion breaks in the midge *Tendipes decorus*. Canad. J. Zool. **35**, 221—263 (1957).

SPERLICH, D.: Modellversuche zur Selektionswirkung verschiedener chromosomaler Strukturtypen von *Drosophila subobscura Coll.* Z. Vererbungslehre **89**, **422—436** (1958). — Experimentelle Beiträge zum Problem des positiven Heterosiseffektes bei der strukturpolymorphen Art *Drosophila subobscura Coll.* Z. Vererb.-Lehre **90**, 273—287 (1959).

STUMM-ZOLLINGER, E.: Vergleichende Untersuchungen über die Inversionshäufigkeit bei *Drosophila subobscura* in Populationen der Schweiz und Südwesteuropas. Z. indukt. Abstamm.- u. Vererb.-Lehre **85**, 382—407 (1952).

TERZAGHI, E., and D. KNAPP: Pattern of chromosome variability in *Drosophila pseudoobscura*. Evolution **14**, 347—350 (1960).

WARD, C. L.: Chromosome variation in *Drosophila melanica*. Univ. Texas Publ. **5204**, 137—157 (1952).

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OCCURRENCE OF B-CHROMOSOMES IN DIPLOID ALLIUM STRACHEYI BAKER AND THEIR ELIMINATION IN POLYPLOIDS

By

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With 10 Figures in the Text

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Introduction

The study of accessory chromosomes has become a fascinating subject of research in recent years. With the discovery of accessory chromosomes in maize by KUWADA (1925) and later by RANDOLPH (1928, 1941), LONGLEY (1921, 1956) and others, investigation in this direction was initiated by several workers (BOSEMARK 1957; DARLINGTON and THOMAS 1941; JANAKI-AMMAL 1940; MÜNTZING 1948, 1958; NYGREN 1957). At present a large number of plant species are known to contain accessory chromosomes. Notable amongst them are species of *Poa, Secale* and a number of other grasses.

In spite of the enormous wealth of data on the occurrence of accessory chromosomes in plants, very little work has been done in India in this direction. It will not be surprising, if in Indian flora which comprises plants of very different climatic zones several species are found with accessories in their chromosome complement. Recently a number of species belonging both to dicotyledonous and monocotyledonous families (SHARMA and BHATTACHARYYA 1960; SHARMA and DEY 1960) have been studied in this laboratory and shown to contain accessory chromosomes.

In connection with a plant collection tour in the Eastern Himalayas, during the summer of 1960, a species of *Allium* has been collected which later, on examination, has been shown to possess accessory chromosomes. In order to work out the cytology of this species in detail, a large number of individuals were collected, brought to the plains and grown in the University Science College compound in Calcutta. Later investigations have revealed certain interesting results which have been outlined in the following text.

Material and Methods

Allium stracheyi BAKER was obtained from the Botanical Gardens in Darjeeling. Young healthy root-tips were pre-treated in a saturated solution of aesculin for 30—45 minutes at 8—10° C. The roots were fixed in acetic alcohol (1 : 2) for 30—40 minutes and heated in aceto-orcein-HCl mixture (1 part NHCl : 9 parts of

2% aceto-orcein) for a few seconds. They were then squashed in 1% aceto-orcein solution on a clean slide. Observations were made: 1. on roots collected at Darjeeling, 2. on roots existing in bulbs brought from Darjeeling to Calcutta and 3. on roots collected from the bulbs after growing them for a few months in Calcutta.

Drawings were made at a table magnification of × 1700 approximately. Microphotographs were taken at a magnification of × 1800 approximately.

Observation

The normal number in the somatic cells as observed from root-tips of plants from Darjeeling, is fourteen. Two chromosomes bear satellites.

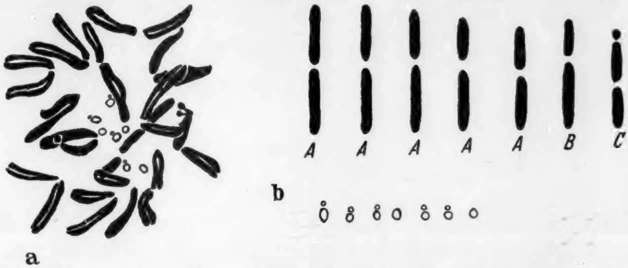


Fig. 1a and b. Normal diploid complement with high number of B-chromosomes. a diploid metaphase. b Idiogram: upper row haploid set of normal chromosomes, lower row B-chromosomes of Fig. 1a. × 1360

Fig. 2. Metaphase of polyploid cell with two B-chromosomes. × 1360

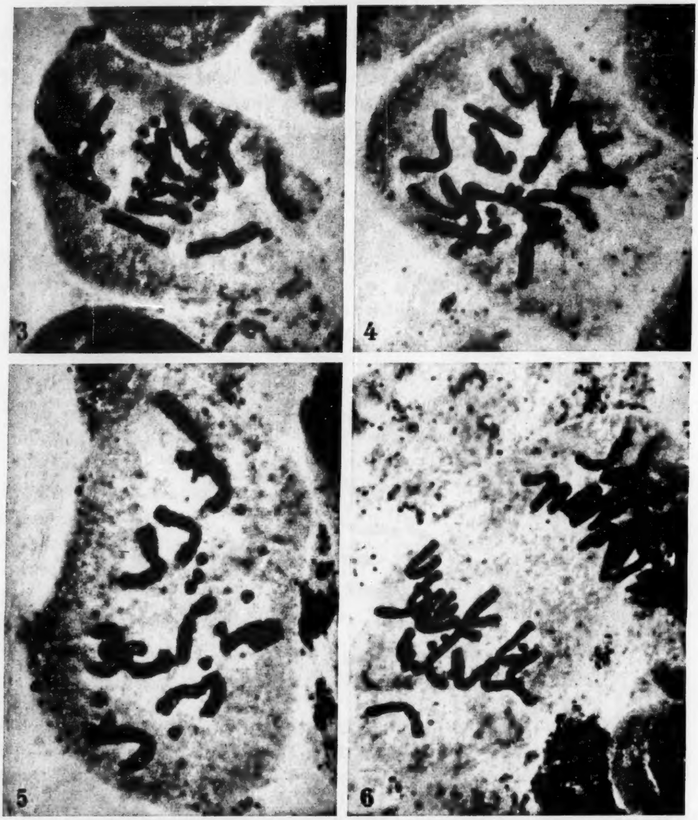
A detailed study of the morphology of chromosomes including the positions of primary and secondary constrictions taken together with the size difference shows the presence of the following types of chromosomes (Fig. 1a and b).

Type A. — Five pairs of long chromosomes with median primary constrictions ranging in length from 21.1 μ to 17.5 μ.

Type B. — One pair of long chromosomes with sub-median primary constrictions 18.7 μ long.

Type C. — One pair of comparatively medium-sized chromosomes with nearly median primary constrictions and with a satellite at the distal end of one arm (17.5 μ in length).

In addition to the normal chromosomes, accessory chromosomes varying from 2—10 in number have been found in all the cells. These accessory chromosomes vary in size (2.8 μ to 1.1 μ) and some of them seem to be provided with a constriction near one end



Figs. 3—6. Diploid cells. 3, 4. Cells with varying number of B-chromosomes. 5. B-chromosomes in a cell with sub-diploid chromosome number. 6. Anaphase showing normal mitotic separation of B-chromatids. × 1800

(Figs. 1—6). They have been detected in both poles of anaphase as well (Fig. 6).

These accessory chromosomes persist even in pollen mother cells and they have been detected in pollen grains as well. In all cases, the number is variable.

A few bulbs were found showing the tetraploid number $4n = 28$, with no 'B'-chromosomes present.

Bulbs grown for some time at Calcutta after having been brought from Darjeeling, all contained polyploid cells (Figs. 7—10). Twenty-eight

Figs. 7—10. Polyploid cells. 7, 8. Cells with one B-chromosome. 9, 10. Cells without B-chromosomes. × 1620

chromosomes were found and occasionally in rare cases even fifty-six as well. In none of the cells the diploid chromosome number could be noticed. The interesting feature of the polyploid cells in root tips is the absence of B-chromosomes (Figs. 9 and 10). So far, only three cells have been recorded in which B-chromosomes occur in polyploid cells. In these

cases, the number of B-chromosomes was reduced to two or one (Figs. 2, 7 and 8).

For confirmation of the results the observations have been repeated in three sets of bulbs collected from Darjeeling in June, August and October, 1960, and in all cases the diploid bulbs of the hills have been found to be converted into polyploids with the elimination of Bs, after having grown in the plains.

Discussion

In the population of *Allium stracheyi*, growing in Darjeeling, both diploids and tetraploids occur. This is an interesting observation especially as the diploids invariably contain B-chromosomes and the tetraploids are conspicuous by their complete absence. The simultaneous occurrence of the two in the same population and the absence of Bs in the tetraploids may be taken to imply that adaptability for diploids with B-chromosomes and for polyploids is similar. The complete replacement of the diploid roots by the tetraploids in the plains is very significant.

With regard to the significance of occurrence of B-chromosome, nothing definite is yet known. After an analysis of different factors, MÜNTZING (1958) has suggested that their continued occurrence in a population is indicative of some selective value. As regards their origin, nothing definite is yet known. DARLINGTON (1955, 1956) was the first to point out that the occurrence of accessory chromosomes is more frequent in diploids than in polyploids. He is of the opinion that the variability of a species may be controlled by the accessory chromosomes and hence in polyploids their presence is not of much significance. MÜNTZING too is inclined towards this idea and recent literature in this direction also provides further support to this statement (vide MÜNTZING 1958).

That the B-chromosomes in the present case do not stand against normal functioning of the cell is apparent in their persistance up to the germ cell stage. They are not only present in the pollen mother cells and take part in the meiotic cycle, but their presence has been detected in the pollen grains also. This behaviour can account for the regular persistence of B-chromosomes in the diploid individuals. Not only are they maintained through vegetative propagation but even in rare cases of seeded reproduction, a fair number is maintained in the progeny. Whether an increased number of B-chromosomes causes a deleterious effect is not clear. MÜNTZING (1943, 1949) in *Secale cereale* observed that though B-chromosomes have a selective value, yet their increased number definitely exerts a deleterious effect on the progeny. Evidently a threshold of their occurrence exists. In the present case, plants have been found to survive showing normal behaviour even when the maximum

number of B-chromosomes in the cell is ten. Whether a further increase is harmful is not yet known.

With regard to the nature of B-chromosomes, it may be recollected that some of them are provided with a minute constriction at one end whereas the others do not show the existence of any such constriction. In the latter, even though cytologically undetectable, centromeres must be present as chromatid movement to the two poles is normal. We have no evidence to state under the present circumstances the mechanism of origin of B-chromosomes noted in *Allium stracheyi*. How far they are heterochromatic in nature, as indicated by tests for heterochromaticity, is yet to be seen.

It has been mentioned before that DARLINGTON first suggested that the B-chromosomes are frequent in diploids and not in polyploids. The present observation advances direct proof of this statement. The simultaneous occurrence as noted here of diploids and polyploids suggests that the adaptability under temperate conditions is the same in both. In all likelihood the fourteen chromosomes in the diploids along with the B-chromosomes are responsible for their adaptability, thus allowing them to compete with the polyploids. The absence of an intermixture and the complete lack of any triploids in the population may be due to the rare seed setting or nonviability of the zygote resulting from the union of a diploid gamate and a haploid one containing B-chromosomes.

Attention is merited by the fact that the populations when brought to the plains gradually become entirely polyploid within a month. The complete conversion of the diploids to polyploids and the simultaneous disappearance of B-chromosomes is extremely interesting. The rare occurrence of polyploid cells with one or two B-chromosomes may suggest that they represent intermediate steps in the process of complete obliteration of B-chromosomes. As the temperature in Darjeeling and Calcutta is very different it appears that shift in temperature and the shock caused thereby may be responsible for polyploidy.

This complete conversion can be effected in two possible ways. Firstly, in the plains, the B-chromosomes being possibly ineffective may degenerate and the degeneration-products in the cytoplasm may help in spindle disturbance effecting polyploidy. Secondly, there is also the likelihood that polyploidy is caused merely by temperature shock and in polyploid cells, B-chromosomes either being ineffective degenerate or can not survive because the increased chromosome complements utilize all the precursors and constituents necessary for normal metabolism thus making the survival of B-chromosomes impossible.

Cases of induction of polyploidy through temperature shocks have already been stated by different authors (comp. RANDOLPH 1932).

Experiments are in progress to study effects in diploid plants when they are kept in temperature controlled chamber in the Cytogenetics Laboratory at Calcutta.

Summary

In a population of *Allium stracheyi* BAKER ($2n = 14$) growing in Darjeeling both diploids and polyploids occur. The diploids contain B-chromosomes varying from 2—10 in number. Polyploids are conspicuous by absence of B-chromosomes. These in diploids are found in the pollen mother cells and also in the pollen, and some are provided with subterminal constriction.

Diploid individuals when brought from Darjeeling to Calcutta (i. e. from temperate to tropical regions) became polyploid within a month and the B-chromosomes were simultaneously lost. In order to confirm this unexpected result, the transfer experiment has been repeated thrice with fresh collections in each case and selection of diploid bulbs after cytological observation. In all cases the result has been the same. In rare cases one or two B-chromosomes were found in the polyploid cells which might represent intermediate steps of the disappearance.

B-chromosomes in diploids possibly help the individual to compete with polyploids by enlarging the adaptive capacity.

The sudden polyploidisation by transfer from the mountains to the plains might have been the result of a shock due to the temperature difference. The high temperature may be deleterious for the reproduction of B-chromosomes, and their degeneration products possibly contribute to cytoplasmic changes and the spindle disturbances which effect polyploidisation.

Literature

BOSEMARK, N. O.: On accessory chromosomes in *Festuca pratensis*. V. Influence of accessory chromosome on fertility and vegetative development. Hereditas (Lund) **43**, 211—235 (1957). — Further studies on accessory chromosomes in grasses. Hereditas (Lund) **43**, 236—297 (1957).

DARLINGTON, C. D.: Natural populations and the breakdown of classical genetics. Proc. roy. Soc. B **145**, 350—364 (1956).

—, and P. T. THOMAS: Morbid mitosis and the activity of inert chromosomes of *Sorghum*. Proc. roy. Soc. B **130**, 127—150 (1941).

JANAKI-AMMAL, E. K.: Chromosome diminution in a plant. Nature (Lond.) **146**, 839—840 (1940).

KUWADA, Y.: On the number of chromosomes in maize. Bot. Mag. (Tokyo) **39**, 227—234 (1925).

LONGLEY, A. E.: Supernumerary chromosomes in *Zea mays*. J. agric. Res. **35**, 769—784 (1937). — The origin of diminutive B-type chromosomes in maize. Amer. J. Bot. **43**, 18—22 (1956).

MÜNTZING, A.: Genetical effects of duplicating fragment chromosomes in rye. Hereditas (Lund) **29**, 91—112 (1943). — Accessory chromosomes in *Poa alpina*. Heredity **2**, 49—61 (1948). — Accessory chromosomes in *Secale* and *Poa*. Proc.

8th Internat. Congr. of Genetics. Hereditas (Lund) Suppl., 402—411 (1949).— Accessory chromosomes. Trans. Bose Res. Inst. **22**, 1—15 (1958). — A new category of chromosomes. Xth Internat. Congr. of Genetics. Canada 1959, p. 453—467.

NYGREN, A: *Poa temoleontis* HELDR., a new diploid species of the section *Bulbophorum* A. et GR. with accessory chromosomes only in the meiosis. Kungl. Lantbrukshögskolans Ann. **23**, 489—495 (1957).

RANDOLPH, L. F.: Types of supernumerary chromosomes in maize. (Abstract.) Anat. Rec. **41**, 102 (1928). — Genetic characteristic of the B-chromosomes in maize. Genetics **26**, 608—631. — Some effects of the high temperature on polyploidy and other variations in maize. Nat. Sci. Proc. **18**, 222—229 (1932).

SHARMA, A. K., and U. C. BHATTACHARYYA: Structure and behaviour of chromosomes in species of *Anthurium* with special reference to accessory chromosome. (1960, in press.)

—, and S. DEY: Karyotype analysis in the genus *Sida* (1960, unpublished).

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CHROMOSOME STUDIES IN THE FAMILY NOTONECTIDAE (CRYPTOCERATA-HETEROPTERA)

By

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With 8 Figures in the Text

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Introduction

So far only seven species of the family *Notonectidae* are known cytologically, all of which belong to the genus *Notonecta* (sub-family *Notonectinae*). These are *N. indica*, *N. shooterii*, *N. undulata*, *N. insulata*, *N. irrorata*, *N. glauca*, and *N. macurata* (BROWNE 1913, 1916; POISSON 1927). The diploid chromosome number of the first three species is 26, whereas the last four possess 24 chromosomes. The sex determining mechanism in all of them is of the typical heteropteran X-Y-male type.

The present communication deals with the chromosome numbers and male meoisis of three species of the genus *Anisops* (sub-family *Anisopinae*). It was found that these species differ from the *Notonecta* species in chromosome number, type of sex chromosome mechanism and type of reduction. Because of this contrast to the cytological characteristics of the *Notonectinae* and the majority of *Heteroptera*, this sub-family presents interesting material for cytological investigations.

Material and Methods

The following three species have been studied: *Anisops niveus* FABR., *A. fiebri* KIRK., and *A. sardea* KIRK. They were collected during the months of July and August from fresh water ponds in which they then can be found in abundance.

Since the testes of the adults do not yield a sufficient number of dividing cells, nymphs of the last two instars were used for the study. The testes were fixed in Sanfelice, Allen's picro-formol and acetic-alcohol. For staining of the 12—15 μ thick sections iron hematoxylin and Feulgen's method were used.

The tips of the ovarioles from female nymphs of *A. niveus* were squashed in aceto-carmine.

Observations

Anisops niveus

Male complement. The diploid chromosome number, as revealed in polar views of spermatogonial metaphase, is 26 (Fig. 1 a). They form a graded series in length. Only the smallest pair is individually distinguishable.

Female complement. Metaphases of diploid cells from the tips of ovarioles contain 28 chromosomes (Fig. 1 b), of which, as in male metaphases, only the smallest pair can be recognized with certainty.

Fig. 1 a and b. *Anisops niveus*, diploid metaphases. a Spermatogonium. b Ovarial cell

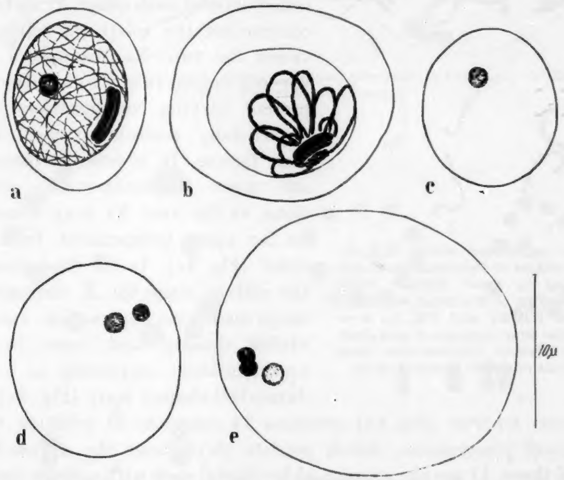

Fig. 2 a—e. *Anisops niveus*, early stages of male meiosis. a Leptotene. b Pachytene. c Early; d middle; e late phase of diffuse stage. In c X_1X_2 invisible, in d visible as a ball-shaped mass, in e as a dumb-bell-shaped body. The round stippled body in a, c—e represents the plasmosome

Sex chromosomes. They are morphologically indistinguishable from the autosomes. A comparison of the diploid chromosome complements in males and females shows that the sex determining mechanism in this species is of an X_1X_2-0 type in the male and of an $X_1X_1X_2X_2$-type in the female.

Male meiosis. Except for the absence of a contraction stage, which normally follows the leptotene stage, the general course of meiosis is

typically heteropteran. The sex chromosomes are visible during leptotene (Fig. 2a) and pachytene (Fig. 2b) as two darkly staining rod-like bodies which lie in close apposition. In the early diffuse stage (Fig. 2c) they lose their staining capacity and become invisible. During the later phase of this stage, however, they reappear as long threads tightly wound into a dense ball-shaped mass (Fig. 2d) in which occasionally some thread sections reveal their duality: they are already split into two chromatids.

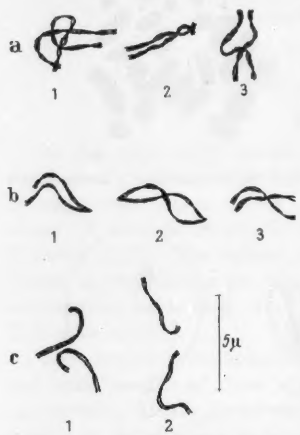

Fig. 3a—c. *Anisops niveus*, X_1X_2 in diverse phases of disentanglement and stretching in later diffuse stages corresponding to the transition stages between Fig. 2d and Fig. 2e. a—c shows the time sequence of uncoiling. *1, 2, 3* denote chromosomes from different nuclei of identical stage

Soon afterwards the Xs unwind themselves and appear as two discrete threads loosely coiled around each other (Fig. 3a). They are divided into two chromatids which are also coiled around each other. With further contraction the relational coiling between the two Xs disappears to a varying degree (Fig. 3b) and they now present varying configurations which superficially resemble typical diplotene figures. It is possible that these are mere heterochromatic associations, as the two Xs may sometimes lie far apart independent from each other (Fig. 3c). In the final phases of the diffuse stage the X elements undergo maximum contraction, lose their visible duality and come in close approximation, appearing as a single dumb-bell-shaped body (Fig. 2e). The diplotene nucleus (Fig. 4a) contains 14 elements in addition to the unstained plasmosome, which persists throughout the diffuse stage. Out of these, 11 are the autosomal bivalents, each with a single chiasma, two are separate m-chromosomes, while the remaining dumb-bell-shaped body represents the two fused X chromosomes. The m-chromosomes are comparatively small, darkly stained and they often lie independent of each other. The nucleus at diakinesis (Fig. 4b) again shows the same 14 elements.

In metaphase I thirteen elements are distinguishable in polar view (Fig. 5a). Of these, 11 autosomal bivalents form a ring enclosing the pair of m-chromosomes in its centre. The X chromosomes are still represented by a single dumb-bell-shaped body which lies outside the spindle area in the cytoplasm slightly off from the equatorial plane.

All the elements have undergone co-orientation except the dumb-bell-shaped one which does not reveal any definite orientation (Fig. 5b).

The two m-chromosomes undergo precocious anaphase disjunction. The dumb-bell-shaped body, formed by the two sex-chromosomes,

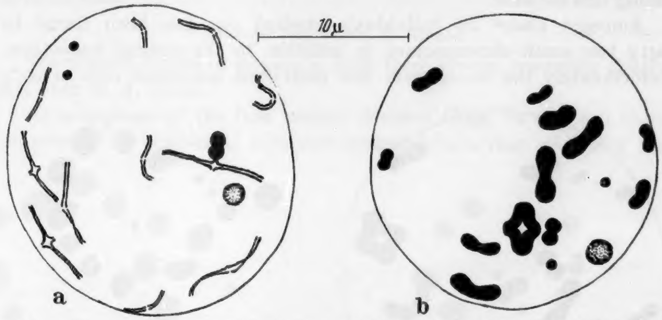

Fig. 4a and b. *Anisops niveus*, later stages of meiotic prophase. a Diplotene. b Diakinesis. Stippled body the plasmosome

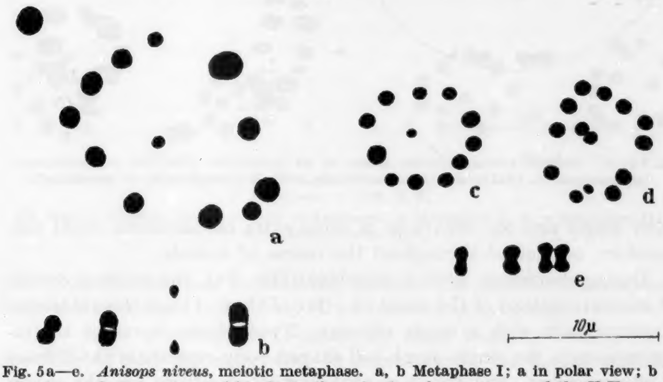

Fig. 5a—e. *Anisops niveus*, meiotic metaphase. a, b Metaphase I; a in polar view; b in side view, only two autosomal bivalents, the pair of m-chromosomes and the X_1X_2 aggregate are shown. c—e Metaphase II; c, d polar views; c plate without X_1X_2; d plate with X_1X_2; e side view, only two autosomal elements are shown, the elements at the extreme right are X_1X_2

does not show any active movement during anaphase I. When the autosomal elements reach their respective poles the X_1X_2-compound is included quite passively into one of the two daughter cells.

Two types of metaphase II plates are the consequence of the first meiotic division, one carrying 12 autosomes only (Fig. 5c), while in the other there are the two sex chromosomes in addition to the 12 auto-

somes (Fig. 5d). Between the X chromosomes lateral connections by chromatic threads are found which are visible from the polar as well as the side views of the spindle (Fig. 5e). All the elements including the two X chromosomes undergo auto-orientation and divide equationally during this division.

Amongst about 50 individuals studied one has been found to carry two small chromosomes in addition to the normal karyotype. Unfortunately the testes from this individual contained cells of only

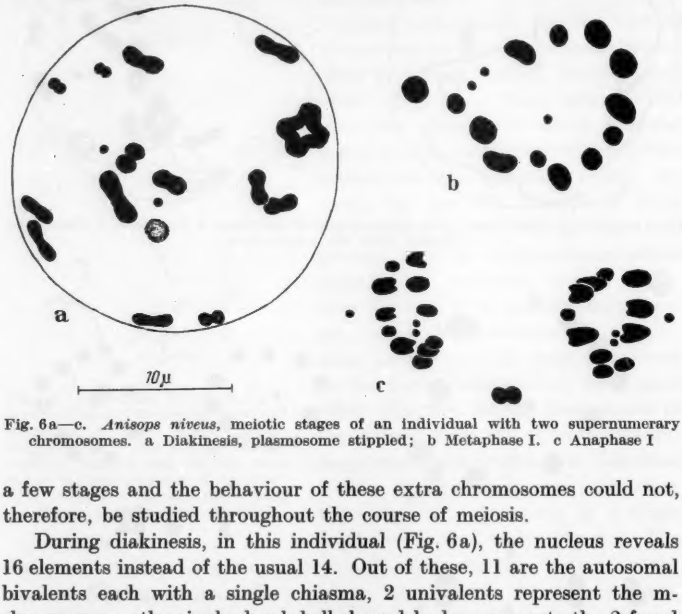

Fig. 6a—c. *Anisops niveus*, meiotic stages of an individual with two supernumerary chromosomes. a Diakinesis, plasmosome stippled; b Metaphase I. c Anaphase I

a few stages and the behaviour of these extra chromosomes could not, therefore, be studied throughout the course of meiosis.

During diakinesis, in this individual (Fig. 6a), the nucleus reveals 16 elements instead of the usual 14. Out of these, 11 are the autosomal bivalents each with a single chiasma, 2 univalents represent the m-chromosomes, the single dumb-bell-shaped body represents the 2 fused X chromosomes, and the 2 small bipartite structures are the supernumerary chromosomes. In metaphase I (Fig. 6b) in this individual 16 elements are present. Of these, 14 form a ring enclosing the pair of m-chromosomes, the two fused sex chromosomes lying in the cytoplasm outside the spindle area. Of the 14 elements in the ring, 10 are the complete bivalents, 2 are autosomal univalents resulting from the precocious separation of the homologues of a bivalent and the remaining 2 small elements are the 2 extra chromosomes which are bipartite and auto-oriented. This is further substantiated by the fact that during anaphase I (Fig. 6c) only 14 elements move to each of the poles, the

sex chromosomes behaving like those in the normal individual. From these observations it may be concluded that the two small bipartite elements at diakinesis, which undergo auto-orientation at metaphase I and thus divide equationally during this division, are the supernumerary chromosomes.

Anisops fiebri and A. sardea

The general course of meiosis in both these species resembles closely that seen in *A. niveus.*

At metaphase of the first meiotic division (Figs. 7a and 8a) there are present 11 autosomal bivalents arranged in a ring, enclosing the

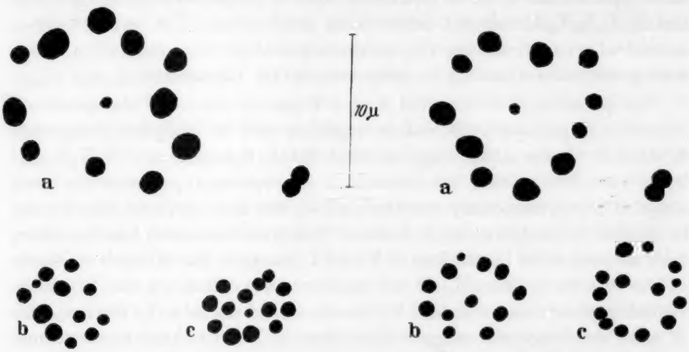

Fig. 7 Fig. 8

Fig. 7a—c. *Anisops fiebri*, meiotic metaphases. a Metaphase I. b, c Metaphase II; b without, c with X_1X_2

Fig. 8a—c. *Anisops sardea*, meiotic metaphases. a Metaphase I. b, c Metaphase II; b without; c with X_1X_2

pair of m-chromosomes in both the species. The two X chromosomes lie in the cytoplasm in the form of a dumb-bell-shaped body.

Two types of metaphase II plates are to be found in both species, one with 12 and the other with 14 chromosomes, the former without and the latter with the X_1X_2 (Figs. 7b, c; 8b, c). Also in these species there are visible chromatic connections between the two closely apposed sex chromosomes.

Discussion

Evolution of the sex chromosomes in the Notonectidae

The sub-family *Notonectinae* is characterised by the presence of a pair of typical post-reductional X and Y chromosomes (Browne 1913 and 1916). The two sex chromosomes differ in their size in all the species except *Notonecta shooterii*, and this difference is large in *N. indica*

(BROWNE 1916). From the fact that the X chromosome in *N. indica* is represented by a single comparatively large element during the spermatogonial divisions and is composed of two parts, one smaller and the other larger, behaving as a unit during the meiosis, BROWNE (1916) regards it as an incipient compound element. She further concludes that this complex is the first link in the chain from a simple X through those cases where X is composed of two elements sometimes combined, sometimes separate, to cases where X consists of two or more elements always separate but acting as a unit in the maturation divisions.

The other sub-family of *Notonectidae*, the *Anisopinae* of which three species have been described above, possesses an $X_1X_2$0-male and $X_1X_1X_2X_2$-female sex-determining mechanism. The two sex chromosomes, separate during the spermatogonial divions, behave as a unit during meiosis remaining in close association throughout.

The presence of an unequal X and Y pair in almost all the species of *Notonecta*, especially in *N. indica*, together with an incipient compound X element in the latter, suggests that the $X_1X_2$0-male and $X_1X_1X_2X_2$-female sex-determining mechanism in *Anisopinae* represents the final stage of the evolutionary transformation, the first steps of which may be similar to conditions in *N. indica*. This transformation has, however, been accompanied by the loss of Y and a change in the division sequence of the two Xs so formed. If the supernumerary element in a few individuals represents a persistent Y, the absence of the latter in the majority of cases would seem to suggest that there its loss has been a recent one.

A similar retention of Y has also been described by WILSON (1910) in *Metapodius*.

DARLINGTON (1940) is of the opinion that the post-reductional behaviour of the heteropteran sex chromosomes is correlated with their precocious division during early prophase of the first meiotic division, which in its turn prevents their pairing at pachytene and finally results in the division of their centromeres at metaphase I. He further states that the pre-reductional behaviour of the sex-chromosomes, wherever present in the group, is due to the reversion of their centromere division cycle to that of the autosomes.

In an earlier communication (JANDE 1960) it has been endeavoured to show that this hypothesis of DARLINGTON is not valid, so far as the pre-reductional behaviour of the sex chromosomes in three species of the *Tingidae* (*Heteroptera*) is concerned. This is primarily due to the absence of any region with special cycle of division in the heteropteran chromosomes and the divided nature of the pre-reductional sex chromosomes even in the early prophase of the first meiotic division in this family. It has been concluded that the change in the behaviour of the

sex chromosomes from post- to pre-reductional concerns the nature of their response to the division centres.

The duplicate nature of the sex chromosomes in the early prophase of the first meiotic division which are otherwise pre-reductional in all the three species of the sub-family *Anisopinae* weakens the validity of DARLINGTON's hypothesis. The mechanics of the pre-reductional sex chromosomes in these three species, however, presents a more complicated situation as far as their later behaviour is concerned. The two Xs in these species resemble the typical post-reductional sex chromosome of *Heteroptera* during the early prophase of the first meiotic division. They have already duplicated by the early diffuse stage and consist of two chromatids along their entire length. During diakinesis the two Xs although duplicated come in close approximation to form a dumb-bell-shaped body. At prometaphase this body is expelled into the cytoplasm outside the spindle area although remaining almost in the equatorial plane of the spindle, and does not undergo any orientation. It is at late anaphase that it is passively included into one of the two daughter secondary spermatocytes. HUGHES-SCHRADER (1948) attributes a somewhat similar expulsion of the X chromosome into the cytoplasm in the mantid *Humbertiella indica* to the delayed response of its kinetochore to the division centres. The same appears to hold true for the dumb-bell-shaped body representing the two X chromosomes in the three species of *Anisops* under discussion. A delay in the response of the kinetochores of these X chromosomes to the division centres leads to their expulsion from the spindle area, lack of orientation at metaphase I and their subsequent passive inclusion into one of the two secondary spermatocyte nuclei.

It may thus be concluded that the change involved in the alteration from the typical post-reductional sex chromosomes in the *Notonectinae* to the pre-reductional ones in the *Anisopinae* concerns the time of kinetochore interaction with the division centres and is not related to the division cycle of the centromeres as postulated by DARLINGTON (1940).

Summary

1. The diploid complement in the males of *Anisops niveus*, *A. fiebri* and *A. sardea* is represented by 26 chromosomes while in the females, studied only in *A. niveus*, it consists of 28 elements.

2. The sex determining mechanism is of the X_1X_20-male and $X_1X_1X_2X_2$-female type in all the three species. The sex chromosomes divide reductionally in the first meiotic division of the male.

3. A pair of m-chromosomes is present in each species while a pair of supernumerary elements occurred in some individuals of *A. niveus*.

4. The X_1X_20-male sex mechanism has probably originated from a simple XY type, similar the one still existing in *Notonecta indica*, by the loss of Y and conversion of the simple X into a multiple one, accompanied by a change in their division sequence.

5. The change from the typical heteropteran post-reductional sex chromosome behaviour to the pre-reductional one, is suggested to be the result of the delay in their kinetochore interaction with the division centres during the first meiotic division, in all the three species of the *Anisopinae*.

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References

Browne, E. N.: A study of the male germ cells in *Notonecta*. J. exp. Zool. **14**, 61—102 (1913). — A comparative study of the chromosomes of six species of *Notonecta*. J. Morph. **27**, 119—161 (1916).

Darlington, C. D.: The genetical and mechanical properties of sex chromosomes. V. *Cimex* and the *Heteroptera*. J. Genet. **39**, 101—137 (1940).

Hughes-Schrader, S.: Expulsion of sex chromosome from the spindle in the spermatocytes of a mantid. Chromosoma (Berl.) **3**, 257—270 (1948).

Jande, S. S.: Pre-reductional sex chromosomes in the family *Tingidae* (*Gymnocerata-Heteroptera*). Nucleus 3, 209—214 (1960).

Poisson, R.: Arch. Zool. exp. géner., **66**, (1927). Quoted from S. Makino, An atlas of chromosome numbers in animals. Iowa 1951.

Wilson, E. B.: Studies on chromosomes. VI. A new type of chromosome combination in *Metapodius*. J. exp. Zool. **9**, 53—78 (1910).

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